REMARKS

Claims 1, 2, 5-8, 11, 12, 14-20, 22-27, 29-33, and 35-55, along with newly presented claims 56-62 are pending in the present application.

Claims 1, 5-8, 11-12, 14-17, 19-20, 22-27, 29-33, 35-40, 48, 50, 51, and 53 have been rejected under 35 USC §103(a) as being unpatentable over Baur et al. Claims 41-47, 49, 52, 54, and 55 have been rejected under 35 USC §103(a) as being unpatentable over Baur et al. as applied to claims 1-3, 5-8, 11-17, 19-20, 22-27, 29-33, and 35-40, and further in view of Haverkos et al.

The Examiner primarily relies on the Baur et al. reference, which discloses, "[t]he food substrate which may be used in the practice of this invention include . . . cereal-based dough products, and nuts. Examples of cereal-based products include pizza doughs, biscuit doughs . . .." ('179 patent, col. 2, lines 5-9). The Examiner has acknowledged that the Baur et al. reference does not "disclose a dough made of potato, a product which emulates a slice of natural food, the thickness of the food substrate, heating in a toaster, a baked product, the product is waffle, pancake o[sic], dusting the food substrate, using dried ingredients for the coating and stabilizer such as carageenan, gum Arabic, guar gum, carboxymethylcellulose." (Office Action mailed May 18, 2004, p. 2). Baur et al. also does not teach a food substrate made from moldable and pliable, but shape retaining dough that is primarily made of potatoes or other such tubers.

Applicants have amended independent claims, 1, 24, 46, 48, 54, and 55 and dependent claim 45 to state that the coating composition forms a substantially clear coating on the substrate after thermal processing. Independent claims 43 and 59 and dependent claim 49 state that the coating composition for a dough comprises a rice flour component (claims 43 and 59) or greater than about 10% rice flour (dependent claim 49).

The present application states, "[e]xamples of particular coatings used on sliced potatoes which yield desirable results in this invention are set forth in commonly owned co-pending Application Nos. 60/180,666 and 60/234,153 (unofficial), which are incorporated herein by reference as fully as those set forth verbatim herein." (Page 2, par. 13). These co-pending patent applications, which were incorporated by reference, specifically disclose that "a

substantially clear coating composition is created." (*See*, for example, p. 4 of 60/180,666). The '666 application also states that "[t]hose seeking substantially clear, smooth and 'invisible' coatings for food products, . . . without producing a dark, opaque, oily, broken, or rough surface texture . . . would prefer the coating of [the present invention]." (*Id.* at pp. 13-14.) Similar statements are made in the 60/234,153 application, which states:

> The food substrate coating industry has for some time attempted to produce coatings for foods such as meat and vegetables, particularly potatoes, in the form of a substantially clear coat. Such "clear coats" are important because of their ability to maintain the original food substrate's natural appearance to the final consumer, while imparting desired characteristics to the reconstituted product, for example increased surface crispness.

(60/234,153 at p. 1).

Later, the Summary of the Invention states that:

> an inexpensive water-dispersible clear coating composition for food substrates may be provided containing concentrations of greater than 10% rice flour which does not reticulate when placed upon food substrates, and which does impart improved color, surface smoothness, crispness, and holding time characteristics to the coated food substrate without sacrificing visual appearance, flavor, and tooth compaction characteristics desirable to end consumers of the final cooked product when reconstituted via gradient heat, microwave energy, or deep-fat frying reconstitution methods . . ..

Id. at pp. 3-4. Accordingly, no new matter has been added by these amendments.

Moreover, the currently pending claims requiring a substantially clear coating are not obvious in view of Baur et al. The Baur et al. reference discloses a wet batter that includes "a texturizing agent in the form of sodium caseinate and in certain applications . . ., added edible fat above and beyond the fat already inherently present in the dry ingredients . . .." ('179 patent, col. 3, lines 7-10). "[I]n applications where a gelatinous uncooked interface is otherwise formed, the texturizing should include added edible fat." (*Id.* at col. 3, lines 30-32) (*emphasis added*). Accordingly, Baur et al. discloses a wet batter that requires sodium caseinate and preferably added fat. The Baur et al. reference repeatedly states that the use of

sodium caseinate in the batter results in a coating with a golden-brown appearance. (*See* Stevens' Dec., pars. 10-12). The patent emphasizes the golden brown appearance of the coated products. (Stevens' Dec., par. 11.) Example 3 of the '179 patent also states that the close-up photographs, Figs. 1 and 2 of the '179 patent, "demonstrate the crisp, crunchy, fracturable coating and golden brown appearance achieved in the caseinate-containing product and the far less desirable appearance and texture achieved in the caseinate-free product." ('179 patent, col. 6, lines 19-27). The batter disclosed in the Baur et al. reference would not function as a substantially clear coating. (Stevens Dec., pars. 13-14). Accordingly, neither Bauer alone nor in combination with Haverkos render the claims requiring the coating be substantially clear obvious.

Furthermore, contrary to the Examiner's assertion, it would not have been obvious "to bake the product if it is desired to reduce the fat content and a baking texture is desired." (Office Action mailed May 18, 2004, at p. 3.) Where a gelatinous uncooked interface is otherwise formed, the Baur et al. patent teaches that its coating contain fat. (Col. 3, lines 30-32.) The cooking conditions of parfrying allow the coating of Baur et al. to congeal/set. (*See* Stevens' Dec., par. 12). However, if the coated substrate of Baur et al. were baked, the much slower cooking or baking would melt the fat and at least a portion of the coating would run off of the substrate prior to the coating setting. (Stevens' Dec., par. 12.)

The Examiner also appears to rely on cols. 3-4 of the '179 patent to Baur et al. for the proposition that baking would be obvious. However, a complete reading of Baur et al. reveals that frying or parfrying is required. The Baur et al. patent describes three preparation methods. First, the batter coated substrate is parfried and then completely cooked. Second, the batter coated substrate is frozen or refrigerated and completely cooked "(as described below)." "Below," the patent states:

> Coated substrates which are not parfried but rather are refrigerated or frozen after coating will require at least partial frying in order to obtain the desired fried appearance in the final product. Thus, such batter-coated substrates may be taken from the refrigerator or freezer and then fully cooked by deep-fat frying or sautéing.

'179 patent, col. 4, lines 27-32 (*emphasis added*). Finally, the patent states that the batter coating can be fully cooked, frozen or refrigerated and then reheated as described below. Immediately thereafter, the '179 patent states that "[i]f the substrate is batter coated but not breaded, it must be parfried or full fried following application of the batter." (*Id.* at col. 4, lines 11-12) (*emphasis added*). Immediately thereafter, the conditions for parfrying and full cooking are described. The reference to any other cooking method, conventional oven, convection oven, microwave oven, by steaming, by deep-fat frying, or sautéing appears when describing how to finalize the parfried product for eating. (*Id.* at col. 4, lines 22-26.) The only other reference to deep-fat frying or sautéing appears in discussing coated substrates that are not parfried but rather refrigerated or frozen after coating, but in the sentence before, the patent requires at least partial frying. (*Id.* at col. 4, lines 27-32.)

Accordingly, the pending claims requiring baking would not have been obvious in view of Baur et al., which in fact teaches away from baking by repeatedly emphasizing that the coated substrates "must be parfried or full fried."

Moreover, while the Examiner admits that Baur et al. do not disclose applying a slurry comprising rice flour and dextrin to a substrate, the Examiner asserts claims requiring a coating composition containing rice flour and dextrin would have been obvious given Baur et al. in view of Haverkos et al., which discloses maltose-free dextrin and rice starch. Applicants respectfully disagree.

As stated in Applicants' March 1, 2004, Response, rice flour is not obvious in view of the disclosure of rice starch and rice starch is substantially soluble while rice flour is substantially insoluble. (Response, p. 12.) Rice starch is more water soluble than rice flour because the starch contains very little protein. (*See* Stevens' Dec., par. 16). Rice starch has about 6% less protein than rice flour and substantially all of the fat is removed from rice starch. (Stevens' Dec., par. 17). Moreover, protein is very important to the structural and functional properties of the rice, and determines the textural (stickiness), pasting capacity, and sensory characteristics of the rice flour. (Stevens' Dec., par. 18.) Rice starch has a soft gel structure, long term stability, higher viscosity buildup at higher temperatures, and a more stable viscosity at lower temperatures than rice flour. (Stevens' Dec., par. 15). Moreover,

rice flour contains not only the carbohydrate fraction (rice starch), but also includes protein, fat, crude fiber, lipids, minerals and vitamin fractions. (Stevens' Dec., par. 17.) The source of caloric components for rice flour and rice starch are as follows:

Rice Flour		Rice Starch	
Protein	about 7%	Protein	about 1%
Carbohydrates	about 90%	Carbohydrates	about 99%
Total Fat	about 4%	Total Fat	about 0%

(Stevens' Dec., par. 17).

The Examiner, in her latest Office Action mailed May 18, 2004, dismissed the solubility differences between rice flour and rice starch because Applicants did not submit evidence to show that rice flour is substantially insoluble while rice starch is substantially soluble. (Office Action, pp. 5-6). Accordingly, Applicants have submitted the Declaration of John F. Stevens, which demonstrates the solubility differences between rice flour and rice starch and other differences at paragraphs 15-19. In particular, the Examiner will note Exhibit 2 of Mr. Stevens' Declaration which pictorially demonstrates the solubility differences.

Accordingly, given the differences between rice starch and rice flour discussed above and in Mr. Stevens' Declaration, absent hindsight, there is simply no teaching to use rice flour in the coating of Haverkos et al. Therefore, Applicants submit the current claims requiring rice flour would not have been obvious over Baur et al. in view of Haverkos.

Applicants have made a concerted effort to place the present application in condition for allowance, and a notice to this effect is earnestly solicited. In the event there are any remaining informalities or any other issues requiring Applicants' assistance, Applicants request the Examiner call Todd A. Van Thomme at (616) 949-9610.

Respectfully submitted,

BRIAN K. DOYLE ET AL.

By: Price, Heneveld, Cooper, DeWitt & Litton, LLP

10/18/2004
Date

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TAV/JAM/LRH/svh/cmu/jrf

Atty. Docket No. ADV12 P-302A
Express Mail No. EV432932211US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	:	1761
Examiner	:	Thuy Tran Lien
Applicants	:	Brian K. Doyle et al.
Appln. No.	:	10/025,403
Filing Date	:	December 19, 2001
Confirmation No.	:	4925
For	:	**COATED FOOD PRODUCTS MADE FROM SHAPED DOUGH SUBSTRATES AND METHOD OF PREPARING SAME**

DECLARATION OF JOHN F. STEVENS

I, John F. Stevens, do hereby declare as follows:

1. I am the Vice President for Research and Development for Advanced Food Technologies, assignee of the present patent application. I graduated from Cornell University with a Food Science Degree in 1970. I have had over 30 years experience in the food science industry. I have had extensive experience specifically in the food coatings area for 14 years, since 1989.

2. From 1989 to 1991, I was the Research and Development Manager for Universal Foods Corporation, where I directed coated French fry developments which resulted in 60 million dollars in additional annual sales for the company. I developed the first clear coat French fry, now having estimated markets sales of over 1 billion pounds per year.

3. From 1991 to 1994, I conducted food coatings research for McCain Foods, Inc.

4. From 1994-1996, I was the Research Manager for Miles Willard Company, directing all frozen and non-snack dehydrated potato development, including the development of a patented clear coat French fry product.

5. From 1996-1999, I was the Director of Northwest Region Technical Services for Newly Weds Foods, Inc. I established, staffed and directed all formula, process, specification, and commercialization of seasoned and clear coat French fry batters for all French fry processors and chain accounts throughout the United States. I developed and

commercialized a signature clear coat french fry for a major processor and for a major national chain account.

6. From 1999 to date, I have served as the Vice President of Research and Development for Advanced Food Technologies. A copy of my resume is attached hereto as Exhibit 1.

7. I am one of the named inventors of U.S. Patent Application Serial No. 10/025,403.

8. I have reviewed the Office Action mailed May 18, 2004, in United States Patent Application Serial No. 10/025,403 and the primary references cited therein, U.S. Patent Nos. 6,288,179 B1 to Baur et al. and 6,265,005 B1 to Haverkos et al.

9. I understand that the Examiner asserts that most of the claims of the application would have been obvious in view of Baur et al. alone or in view of Haverkos et al.

10. The abstract of the Baur et al. reference specifically states that the food product has a "crisp texture, golden brown appearance and fresh fried taste of conventionally fried foods."

11. The "Summary of the Invention" also states:

> the present invention is directed to battered and battered/breaded foods with a crisp texture, a golden brown appearance and a fresh fried taste.

Throughout the remainder of the Baur et al. patent are references to the resultant golden brown appearance of the coated products. Example 2 even states that "batter without fat or sodium caseinate showed significantly inferior texture and undesirable interface as compared to those containing the caseinate/fat texturizing agent."

12. The Baur et al. patent is directed toward the use of a particular, non-gelling milk protein, sodium caseinate. The batter of the Baur et al. patent should have a total solids level of at least 30% by weight. As stated in col. 3, the batter also contains added edible fat at a level of about 2% to 4% by weight <u>in the final hydrated batter</u>. Therefore, the amount of fat in the dry mix is about three times more, from 6.67% to 13.34%. The Baur et al. patent also only describes parfrying or full frying. The patent even states that the coated substrates

that are not parfried but rather are refrigerated or frozen after coating will require at least partial frying in order to obtain the desired fried appearance in the final product. Every example describes parfrying a product. This makes sense in the case of the batter described in the Baur et al. patent. The batter must be fried in oil to congeal/set the batter on the substrate. If a product coated with the batter of the Baur et al. patent was baked and not fried, the slower cooking rate would cause the fat in the batter to melt and some, if not large portions, of the coating would run off the substrate and the coating would not set.

13. Based on my review, the batter of the Baur et al. reference is designed to provide a golden-brown appearance, a characteristic undesired in a clear coat composition, which is substantially clear upon thermal processing.

14. In my opinion, because of the presence of sodium caseinate, the batter disclosed in the Baur et al. reference would not function as a substantially clear coating composition upon thermal processing.

15. Rice starch has a soft gel structure, long term stability, higher viscosity build up at higher temperatures, and a more stable viscosity at lower temperatures than rice flour.

16. Rice starch is more water soluble than rice flour because it contains very little protein (*see* Exhibit 2).

17. Rice flour contains not only the carbohydrate fraction (rice starch), but also includes protein, fat, crude fiber, lipids, minerals and vitamin fractions. The source of caloric components for rice flour and rice starch are generally the following:

Rice Flour		Rice Starch	
Protein	about 7%	Protein	about 1%
Carbohydrates	about 90%	Carbohydrates	about 99%
Total Fat	about 4%	Total Fat	about 0%

18. Protein is very important to the structural and functional properties of the rice, and determines the textural (stickiness), pasting capacity, and sensory characteristics of the rice flour (*see* Exhibit 3, *Rice: Chemistry and Technology*, 3rd ed. at p.143, 152).

19. I took 50 grams of rice flour and stirred it into 150 grams of water at 70° F. I then placed the rice flour mixture into a 250 ml graduated cylinder and let it stand. Likewise,

demonstrated as much less soluble (only 120 ml) than the rice starch (174 ml). After 4 hours there was little change in solubility difference. Rice flour was 110 ml while the more soluble rice starch was 168 ml. The attached photos (Exhibit 2) demonstrate the difference visually.

20. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true, and further, these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 USC §1001, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

October 18, 2004
Date

John F Stevens
John F. Stevens

JOHN STEVENS

200 Cobblestone Lane • Idaho Falls, Idaho 83404 • (208) 529-9027

OBJECTIVE

Management position in food-related product development

PROFESSIONAL SUMMARY

Director with 28 years of product management experience. Demonstrated ability to structure innovative solutions to complex problems that allow for increased profits. Proven ability to develop quality teams that motivate others to their peak performance and reduce execution time.

CAREER HISTORY

NEWLY WEDS FOODS, INC. 1996 – 1999
Idaho Falls, Idaho

DIRECTOR, NORTHWEST REGION TECHNICAL SERVICES

- Established, staffed, and directed all formula, process, specification, and commercialization of seasoned and clear coat french fry batters for all french fry processors and chain accounts throughout the United States.
- Worked directly with all french fry processors to define, develop, and implement process improvements, and introduce new and cost reduced products to their lines.
- Salvaged failing business at key customer by demonstrating technical expertise with flours, starches, seasonings, processing capability, and implementing process savings in excess of $1,000,000 per year.
- Developed and commercialized a signature clear coat french fry for a major processor and for a major national chain account.
- Developed patent-pending process for maintaining light colored fried potato products throughout the year.

MILES WILLARD COMPANY 1994 – 1996
Idaho Falls, Idaho

RESEARCH MANAGER

- Directed all frozen and non-snack dehydrated potato development, creating 8 new product opportunities and bringing on board two new clients on a royalty-paying basis.
- Developed patented clear coat french fry product and patent-pending processes for reduced fat coated fries, and for improved chopped & formed product.

McCAIN FOODS, INC. 1991 – 1994
Frozen Foods Division – Othello Washington

DIRECTOR, TECHNICAL SERVICES

- Directed all research, quality assurance, nutrition, specification, labeling, and internal sensory for the USA multi-plant operation.
- Commercialized signature french fry line, resulting in $30,000,000 sales.
- Commercialized first flavored marinade french fry line valued at $10,000,000, obtaining patent.

UNIVERSAL FOODS CORPORATION 1989 – 1991
Frozen Foods Division – Twin Falls, Idaho

R&D MANAGER, NEW PRODUCTS

- Directed coated french fry developments and internal sensory resulting in $60,000,000 additional sales.
- Developed first clear coat french fry now having estimated market sales of over 1 billion pounds per year.
- Instituted cost reduction programs resulting in $4,000,000 savings per year.

JOHN STEVENS

STEVENS LABORATORIES, INC. 1988 – 1989
Rochester, New York

GENERAL MANAGER

- Took over family business of food & wastewater analyses from father who was retiring. Computerized and streamlined operations.

THE PILLSBURY COMPANY 1985 – 1988
Minneapolis, Minnesota

- Directed all dehydrated potato maintenance valued at $150,000,000.
- Created concept and development of marketed microwave potato specialty line valued at $20,000,000.
- Headed team for the development of 12 marketed food service bakery toppings valued at $8,000,000.
- Developed and implemented cost reduction programs amounting to more than $1,000,000, achieving an award for outstanding cost reduction contribution.
- Developed a sulfite program that established benchmarks for the FDA and resulted in identifying the ability to significantly reduce use levels.

THE R. T. FRENCH COMPANY 1970 – 1985
(Potato Division acquired by The Pillsbury Company in 1985)
Rochester, New York / Idaho Falls, Idaho

MANAGER, FOOD SERVICE BUSINESS DEVELOPMENT 1984 – 1985

- Identified new business areas resulting in a $10,000,000 development strategy.
- Built the food service laboratory and directed programs requiring identifying and implementing new package design / copy, resulting in increased product marketability.
- Developed and launched a new concept of potato in a pouch resulting in $15,000,000 sales.
- Worked directly with distributorships, national account managers, brokers, and ad / creative design houses.

MANAGER, PRODUCT RESEARCH & DEVELOPMENT 1978 – 1984

- Built the research facility and directed new product and sensory programs resulting in $32,000,000 additional retail sales per year and $40,000,000 food service sales per year.
- Constructed and directed cost reduction programs resulting in $6,000,000 savings.
- Oversaw development of specifications, nutrition labeling, and package design.

SUPERVISOR, TECHNICAL SERVICES 1976 – 1978
SENIOR SCIENTIST 1974 – 1976
FOOD SCIENTIST 1970 – 1974

- Responsible for the development of the Automash Potato Dispenser system, and sales personnel training resulting in $8,000,000 sales per year. Developed specialty blend mashed products and food service casseroles for major chain accounts resulting in $50,000,000 sales. Developed wet and dry system blends such as mustard, ketchup, spaghetti, barbecue, and specialty sauces.

EDUCATION

Cornell University, Ithaca, New York **Bachelor of Science Degree, June 1970**
The College of Food and Dairy Science (Food and Dairy Science)

REFERENCES FURNISHED UPON REQUEST

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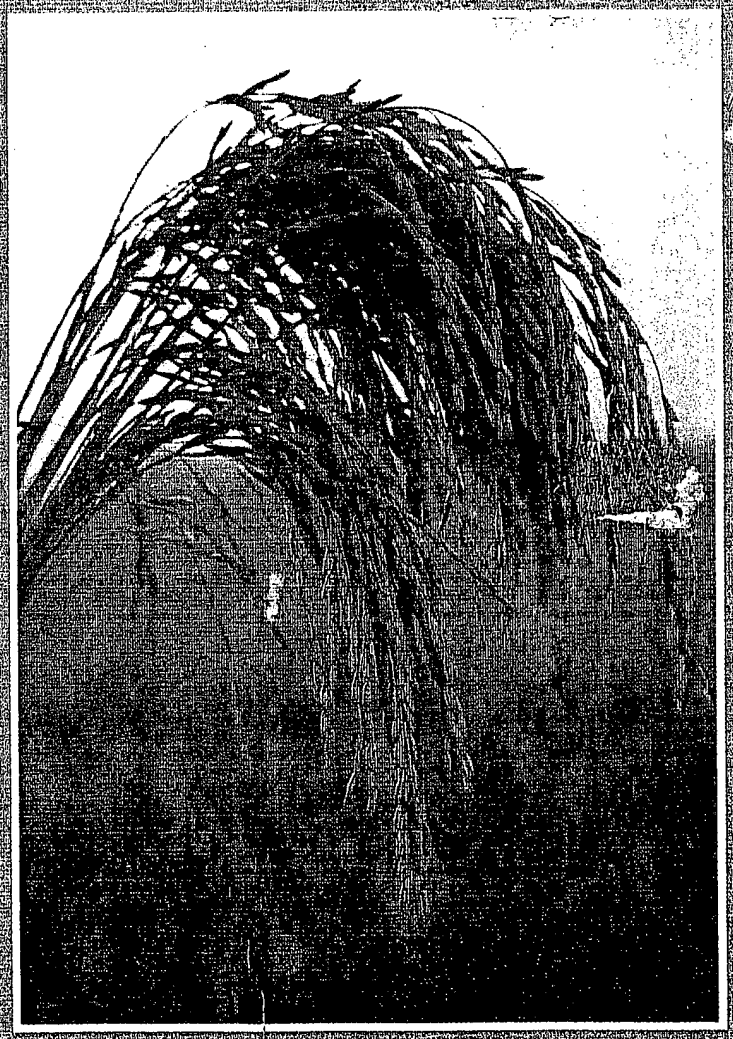

Elaine T. Champagne, editor

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Chemistry and Technology
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Edited by

Elaine T. Champagne

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digestible), and 3) crystalline protein bodies (2–3.5 μm in diameter, crystalline, completely pepsin-digestible, and partially pronase-digestible). The central starchy endosperm has only the large spherical protein bodies. When the protein bodies are isolated, they have a tendency to fuse, supporting the idea that they contain a single membrane (Fig. 9F) (Juliano and Bechtel, 1985).

COMPOSITION OF THE RICE GRAIN AND ITS MILLING FRACTIONS

Proximate Analysis of Rough Rice and Its Fractions

The composition and properties of rough rice and its fractions are subject to varietal, environmental, and processing variability. A wide range of values is evident for all milling fractions (Tables 1–3).

Hull

The rice hull represents 20% of the rough rice grain and is composed of approximately 20% silica. This high concentration of silica is located in the outer layers and, with the lignin (9–20%), provides physical protection of the grain from attack by insects and fungi. The silica is amorphous and has been recovered by various methods (e.g., acid leaching and gasification) (Luan and Chou, 1990; Chakraverty and Kaleemullah, 1991). Cutin, a polymer of long-chain hydroxymonocarboxylic acid, a water-repellent material covering the outer layers of rice hulls, makes up 2–6% of the hull (Luh, 1991). The major carbohydrates are cellulose, crude fiber, and hemicellulose (Table 1). Unlike the bran and endosperm fractions, the hulls are void of starch. Protein and lipid contents are low. Hull lipids from a Brazilian variety were shown to have a content of unsaponifiable matter four times higher than those from the bran

TABLE 1
Range of Mean Proximate Analysis and Content (%) of Organic Fractions of Rough Rice and Its Milling Fractions at 14% Moisture[a]

Nutrient	Rough	Brown	Milled	Hull	Bran	Embryo	Polish
Protein (N x 5.95)	5.8–7.7	4.3–18.2	4.5–10.5	2.0–2.8	11.3–14.9	14.1–20.6	11.2–12.4
Crude fat	1.5–2.3	1.6–2.8	0.3–0.5	0.3–0.8	15.0–19.7	16.6–20.5	10.1–12.4
Crude fiber	7.2–10.4	0.6–1.0	0.2–0.5	34.5–45.9	7.0–11.4	2.4–3.5	2.3–3.2
Crude ash	2.9–5.2	1.0–1.5	0.3–0.8	13.2–21.0	6.6–9.9	4.8–8.7	5.2–7.3
Available carbohydrates	64–73	73–87	77–89	22–34	34–62	34–41	51–55
Starch	53.4	66.4	77.6	1.5	13.8	2.1	41.5–47.6
Neutral detergent fiber	16.4–19.2	2.9–3.9	0.7–2.3	65.5–74.0	23.7–28.6	13.1	–
Pentosans	3.7–5.3	1.2–2.1	0.5–1.4	17.7;18.4	7.0;8.3	4.9;6.4	3.6–4.7
Hemicelluloses	–	–	0.1	2.9;11.8	9.5–16.9	9.7	–
Cellulose	–	–		31.4–36.3	5.9–9.0	2.7	–
1,3:1,4 β-glucans	–	0.11	0.11	–	–	–	–
Polyuronic acid	0.6	–	–	–	1.2	0.4	–
Free sugars	0.5–1.2	0.7–1.3	0.22–0.45	0.6	5.5–6.9	8.0–12	–
Lignin	3.4	–	0.1	9.5–18.4	2.8–3.9	0.7–4.1	2.8
Energy (kJ/g)	15.8	15.2–16.1	14.6–15.6	11.1–13.9	16.7–19.9		17.9

[a] Adapted from Table 1 in Juliano and Bechtel (1985); Gomez (1979); Eggum et al (1982); Pedersen and Eggum (1983); and Bett-Garber et al (2001).

and caryopsis (Hartman and Lago, 1976). The unsaponifiable matter consisted of hydrocarbons, alcohols, and sterols, with the sterols consisting of 50% β-sitosterol, 20–40% campesterol, 10–20% stigmasterol, and 2–3% cholesterol. Differences in fatty acid composition were observed relative to the caryopsis, with the presence of 2–3% saturated C-22 and C-24 acids and a lower proportion of unsaturated acids.

TABLE 2
Range of Mean Content of Elements of Rough Rice and Its Milling Fractions[a]

Element	Rough	Brown	Milled	Hull	Bran	Embryo	Polish
Macroelements[b]							
Calcium	0.1–0.8	0.1–0.5	0.1–0.3	0.6–1.3	0.3–1.2	0.2–1.0	0.5–0.7
Magnesium	0.6–1.5	0.2–1.5	0.2–0.5	0.3	5–13	4–13	6–7
Phosphorus	1.7–3.9	1.7–4.3	0.8–1.5	0.3–0.7	11–25	10–21	10–22
Phytin phosphorus	1.8–2.1	1.3–2.7	0.3–0.7	0	9–22	7–16	12–17
Potassium	1.5–3.7	0.6–2.8	0.7–1.3	1.5–7.5	10–20	11–15	7;11
Silicon	10.8	0.6–1.4	0.1–0.4	64–95	3–5	0.4–0.9	1.1;1.6
Sulfur	0.4–1.6	0.3–1.9	0.8	0.4	1.7	–	1.6
Microelements[c]							
Aluminum	26–540	0.3;26	0.1;2.2	52	200	–	–
Cadmium	–	0.02–0.16	0.025	–	–	–	–
Chlorine	500–800	210–560	200–300	860	66	1200	–
Cobalt	–	0.03–0.04	0.017	–	–	–	0.05
Copper	2–11	1–6	2–3	30–39	9–34	9–34	5–26
Iodine	–	0.03	0.02	–	–	–	–
Iron	14–60	2–52	2–28	39–95	86–430	60–180	43–155
Manganese	17–94	2–36	6–17	100–290	95–230	91–120	–
Molybdenum	–	0.3–1.0	1.4	–	–	–	–
Nickel	–	0.2–0.5	0.14	–	–	–	–
Selenium	–	0.3	0.3	–	–	–	–
Sodium	53–810	17–340	5–86	67–826	71–335	139–636	trace–138
Tin	–	–	<1.1	0	21	–	–
Zinc	1.7–31	6–28	6–23	9–40	43–258	57–258	17;60

[a] Adapted from Table 3 in Juliano and Bechtel (1985).
[b] mg/g at 14% moisture.
[c] μg/g at 14% moisture.

TABLE 3
Range of Mean Vitamin Content (μg/g at 14% moisture) of Rough Rice and Its Milling Fractions[a]

Vitamin	Rough	Brown	Milled	Hull	Bran	Embryo	Polish
Retinol (A)	0–0.08	0–0.11	0–trace	0	0–3.6	0–1.0	0–0.9
Thiamin (B_1)	2.6–3.3	2.9–6.1	0.2–1.1	0.9–2.1	12–24	17–59	3–19
Riboflavin (B_2)	0.6–1.1	0.4–1.4	0.2–0.6	0.5–0.7	1.8–4.3	1.7–4.3	1.7–2.4
Niacin (nicotinic acid)	29–56	35–53	13–24	16–42	267–499	28–83	224–389
Pyridoxine (B_6)	4–7	5–9	0.4–1.2	–	9–28	13–15	9–27
Panthothenic acid	7–12	9–15	3–7	–	20–61	11–28	26–56
Biotin	0.04–0.08	0.04–0.10	0.01–0.06	–	0.2–0.5	0.3–0.5	0.1–0.6
Inositol, total	800	1000	90–110	–	4000;8000	3200;5500	3700;3900
Choline, total	760–980	950	390–880	–	920–1460	1700;2600	860–1250
p-Aminobenzoic acid	0.3	0.3	0.12–0.14	–	0.65	0.9	0.6
Folic acid	0.2–0.4	0.1–0.5	0.03–0.14	–	0.4–1.4	0.8–4.1	0.9–1.8
Cyanocobalamin (B_{12})	0–0.003	0–0.004	0–0.0014	–	0–0.004	0–0.01	0–0.003
α-Tocopherol (E)	9–20	9–25	trace–3	–	26–130	76	54–86

[a] Reprinted from Table 4 in Juliano and Bechtel (1985).

In addition to the protective role, rice hulls exhibit antioxidative defense systems that protect the ability of the mature rice seed to germinate during storage (Osawa et al, 1992). Isovitexin, an antioxidant, has been isolated from indica rice hulls (Osawa et al, 1992). Phenolic-containing fractions isolated from hulls exhibited antioxidant activity stronger than that of α-tocopherol (Ramarathnam et al, 1986, 1988).

Ferulic, vanillic, *p*-hydroxybenzoic, *p*-coumaric, and indolacetic acids and *p*-hydroxybenzaldehyde were identified in rice hull extracts that inhibited germination (Mikkelsen and Sinah, 1961). Hull extracts from some of 91 cultivars were demonstrated to have significant allelopathic potential in inhibiting barnyard grass germination, seedling growth, weight, and caloric content (Ahn et al, 2000).

Compounds that regulate plant growth also have been isolated from rice hulls. One such compound was nicotinamide, a plant-growth regulator (Takeuchi et al, 1975). A chitinase specific to rice hulls also has been isolated and characterized (Baek et al, 2001). Chitinases play roles in defending plants (Boller, 1988), regulating plant development (De Jong et al, 1992), and signal transduction (Roche et al, 1991). Table 2 lists the mineral content of hulls. These data are from independent studies in southeast Asia and the United States and were reported in the previous edition (1985) of this monograph. Marr et al (1995) examined the uptake of elements by brown rice, hulls, and straw (Table 4). Hulls generally removed smaller amounts of minerals; the concentrations of phosphorus, potassium, magnesium, sulfur, zinc, copper, and molybdenum were considerably lower in them than in the brown rice or straw. Hulls removed higher amounts of calcium, manganese, and sodium than brown rice.

TABLE 4
Removal of Elements by an Average Amaroo Crop Yielding 10 t ha^{-1} Paddy[a,b]

Nutrients Removed	In 8.4 t ha^{-1} Brown Grain[c]	In 1.6 t ha^{-1} Hulls[c]	In 11 t ha^{-1} Straw[d]	Total Nutrients Removed	Nutrient Harvest Index[e] (at 10 t ha^{-1}) (%)
kg ha^{-1}					
Nitrogen	99	3.7	70	172.7	57
Phosphorus	24	0.3	5.5	29.8	81
Potassim	20	9	243	272	7
Magnesium	9	0.6	17	26.6	34
Sulfur	8	0.5	6.6	15.1	53
Calcium	0.8	1.4	26	28.2	3
Manganese	0.3	0.6	6.7	7.6	4
Sodium	0.2	0.3	30.8	31.3	1
Aluminum	0.1	0.1	7.1	7.3	1
Iron	0.1	0.1	2.5	2.7	4
g ha^{-1}					
Zinc	134	25	297	456	29
Copper	34	2	40	76	45
Boron	29	20	94	143	20
Molybdenum	5.9	1.4	29	36.3	16
Nickel	3.4	0.6	0.6	4.6	74

[a] Reprinted, with permission, from Marr et al (1995).
[b] A crop that yields 10 t ha^{-1} of paddy will, on average, have 8.4 t ha^{-1} brown rice, 1.6 t ha^{-1} hulls, and produce 11 t ha^{-1} straw.
[c] Expressed at 14% moisture.
[d] Expressed at 0% moisture.
[e] Nutrient harvest index = amount in grain (kg ha^{-1})/amount in grain, hulls, and straw (kg ha^{-1}) × 100/1.

Vitamin contents are listed in Table 3. Values have been reported for the B vitamins (thiamin, riboflavin, and niacin) (Hsu and Luh, 1980).

Bran

Commercial bran makes up 10–15% of rough rice and may contain varying proportions (up to 20%) of polish. The germ usually is included in the bran fraction unless removed by sieving.

Rice bran is an excellent source of protein (12–15%) and lipids (15–20%). Chapters 6 and 7 discuss these components in detail. The major proteins in bran are albumin and globulin, with the albumin-globulin-prolamin-glutelin ratio being 37:36:5:22 for a set of six samples (Cagampang et al, 1966). Rice proteins are considered to be hypoallergenic (Helm and Burks, 1996). However, proteins with molecular masses of 14–16, 26, 33, and 56 kDa found in the bran and endosperm have been shown to be potentially allergenic, based on immunoglobulin E binding (Urisu et al, 1991). The homologous group of 14- to16-kDa protein recognized by 90–95% of patients with rice allergy are α-amylase/trypsin inhibitors (Izumi et al, 1992, Adachi et al, 1993, Nakase et al, 1996). The 26-kDa protein is an α-globulin (Limas et al, 1990). The 33-kDa protein is a novel type of plant glyoxalase I (Usui et al, 2001). The 56-kDa protein has not been identified. More than 150 rice varieties in Japan were screened for the 16-kDa protein and all varieties tested contained nearly the same amount (Adachi et al, 1995). In contrast, some varieties in other Asian countries contained little or none of the allergen.

A thiamin-binding protein in rice bran and germ has been isolated and characterized (Nishino et al, 1980; Nishimura et al, 1984; Shimizu et al, 1996). A fibronectin-binding protein with cell adhesion activity for animal tumor cells also has been identified (Shoji et al, 2001) The protein has an amino-terminal acid sequence identical to that of a putative mature form of hydroxyproline-rich glycoprotein. A distinct feature of the amino acid composition of the protein was the high content of hydroxyproline and proline, representing ~45% of the total amino acids.

Nonstarch lipids are the most abundant form of lipid in bran and are found in the aleurone, subaleurone, and germ. They are composed primarily of neutral lipid with lesser amounts of glycolipids and phospholipids. Starch lipids are a much lesser component and are primarily in the endosperm (Choudhury and Juliano, 1980a). Minor lipid components are sterols, tocol, tocotrienols, and waxes.

The typical composition of extracted crude rice bran oil is 68–71% triglycerides, 2–3% diglycerides, 5–6% monoglycerides, 2–3% free fatty acids, 2–3% waxes, 5–7% glycolipids, 3–4% phospholipids, and 4% unsaponifiables (McCaskill and Zhang, 1999). The phospholipids predominantly include phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. Table 5 compares the fatty acid composition of rice bran oil extracted from U.S. varieties with oil from selected oilseeds (McCaskill and Zhang, 1999).

The sterol composition of rice bran was found to consist of three 4,4-dimethylsterols, seven 4-monomethylsterols, 14 4-desmethylsterols, and 10 minor sterols (Narumi and Takatsuto, 2000). Oryzanol, a phytosterol esterified to ferulic acid, is found at the 2% level or higher in crude rice bran oil. Its health-promoting proper-

ties include plasma cholesterol reduction (Lichtenstein et al, 1994), reduced cholesterol absorption (Rong et al, 1997), decreased aortic fatty streaks (Rong et al, 1997), increased fecal bile acid excretion (Seetharamaiah and Chandrasekhara, 1990), and inhibition of platelet aggregation (Seetharamaiah et al, 1990).

Crude rice bran oil contains tocotrienols, at ~1,000 ppm, which are antioxidants with health-protective benefits in prevention of cancer and cardiovascular disease (Tomeo et al, 1995; Nesaretnam et al, 1998). The wax component of bran contains policosanols, a collection of C-24 to C-34 primary alcohols. Policosanols from sugar cane wax, which are composed of 70% octacosanol (C-28), have been demonstrated to effectively lower low-density lipoprotein (LDL) cholesterol and increase serum high-density lipoprotein (HDL) cholesterol, thus improving the HDL-LDL ratio (Menendez et al, 1994; Rodriguez-Echenique et al, 1994; Batista et al, 1996; Zardoya et al, 1996). Rice bran wax contains less octacosanol (20–44%); its effectiveness in lowering LDL cholesterol has not been demonstrated clinically to date.

Rice bran lipases are a major cause of deterioration of oil in the bran. Lipases are localized in the testa layer and, to a smaller extent, in the pericarp layers (Sastry et al, 1977). The primary enzyme has a pH optimum of 7.5 and temperature optimum of 30°C (Rajeshwara and Prakash, 1995). It has a molecular mass of 30 kDa and is composed of at least two subunits. Rice bran lipase II has a molecular mass of 33 kDa, pH optimum at 7.5–8.0, and temperature optimum of 27°C (Aizono et al, 1976). A thermally stable lipase was recently identified and found to be a glycoprotein of 9.4 kDa (Bhardwaj et al, 2001). The lipase showed a maximum activity at 80°C and pH 11.0.

Lipoxygenase, which is localized in the bran, catalyzes the oxidation of polyunsaturated fatty acids containing a 1,4-pentadiene structure, such as linoleic and linolenic acids, into conjugated hydroperoxy fatty acids, which, in turn, are converted into various volatile compounds. Three isozymes have been characterized (Yamamoto et al, 1980; Ida et al, 1983; Ohta et al, 1986; Zhang et al, 1996). Of 108 varieties from China's Yunnan province, 22 varieties lacked lipoxygenase-3 (Ise et al, 2002). Most of these were japonica upland rice varieties that displayed early heading, round grains, lower amylose content in the endosperm, and glabrous leaves and hulls. During storage, the increase in off-flavor volatile compounds was found to be one-third to one-fifth as much in rice without lipoxygenase 3 compared with the increase in rice containing lipoxygenase 3 (Suzuki et al, 1999).

Starch is found in the aleurone layers of bran in the developing grain, but it disappears with grain maturity (Del Rosario et al, 1968). It is absent in the pericarp and seed coat (Luh et al, 1991). Commercial bran, however, also contains starch contributed by endosperm and germ, with values ranging from 10 to 55%. Bran is high in the nonstarch carbohydrates, cellulose and hemicelluloses. Mod et al (1978, 1979) determined that the water- and alkali-soluble hemicelluloses in the bran contain the sugars rhamnose, arabinose, xylose, mannose, galactose, and glucose, as well as some protein, hexuronic acid, and ferulic acid. Arabinose was the predominant sugar, with the arabinose-to-xylose ratio ranging from 2.8:1 to 5.4:1. The arabinose-xylose ratios for alkali-soluble hemicelluloses ranged from 0.9:1 to 1.1:1.

TABLE 5
Comparison of the Fatty Acid Composition[a] of Rice Bran Oil Extracted from U.S. Varieties with Oil from Selected Oilseeds[b]

Fatty Acid	Rice Bran	Peanut	Soybean	Cottonseed
Myristic (14:0)	0.2	–	0.2	0.8
Palmitic (16:0)	15.0	8.1	10.7	27.3
Stearic (18:0)	1.9	1.5	3.9	2.0
Oleic (18:1)	42.5	49.9	22.8	18.3
Linoleic (18:2)	39.1	35.4	50.8	50.5
Linolenic (18:3)	1.1	–	6.8	–
Arachidic (20:0)	0.5	1.1	0.2	0.3
Behenic (22:0)	0.2	2.1	0.1	–

[a] Percentage.
[b] Reprinted, with permission, from McCaskill and Zhang (1999).

The mineral content of bran is listed in Table 2. With the exception of silicon, the levels of the elements are much higher in the bran and germ than in the other milling fractions. Distribution in the bran and embryo are similar. Approximately 90% of the phosphorus in the bran is phytic phosphorus. Phytic acid, myo-inositol-1,2,3,4,5,6-hexakis (dihydrogen phosphate), strongly chelates minerals (e.g., iron and zinc) and complexes with proteins, with and without mineral mediation, to reduce mineral bioavailability and alter protein functionality (Cheryan, 1980; Morris, 1986; Clydesdale, 1988; Erdman and Poneros-Schneier, 1989; Lopez et al, 2002). Besides having a role as an antinutritional factor, phytic acid also has beneficial effects on health, acting as an antioxidant (Minihane and Rimbach, 2002) and anticancer agent (Shamsuddin, 2002; Steer and Gibson, 2002).

Potassium and magnesium phytates are the principal phytate salts in rice bran (Tanaka et al, 1973). Ogawa et al (1975) found aleurone particles embedded with these salts and covered with a protein and carbohydrate coat, which may have a role in the dephosphorylation of phytic acid during germination of the grain.

Recent research has been directed at reducing phytate content in rice through genetic and molecular technologies. A low-phytic-acid mutant (*Ipa-1-1*) in rice, which reduces the phytic acid portion of the grain phosphorus from 71 to 39% and increases the inorganic portion of the grain phosphorus from 5 to 32%, has been isolated (Larson et al, 2000; Raboy et al, 2002). The *Ipa* allele is nonlethal and is being used to breed low-phytate rice.

As with the distribution of minerals, the vitamins are present at much higher levels in the bran and germ than in the other milling fractions (Table 3). Rice contains little or no vitamin A, C (ascorbic acid), or D. Some of the vitamins, such as riboflavin and niacin, are not in a completely free form. The values for inositol and choline are total values, because inositol is mainly in the form of phytin, and choline is mainly in the phospholipids lecithin and lysolecithin.

Commercial rice bran has a sweet, cereal-like aroma when fresh. During storage, objectionable odors develop as a result of lipid degradation through lipolytic hydrolysis and oxidation. Fujimaki et al (1977) and Tsugita et al (1978) identified 170 compounds contributing to the aroma. 4-Vinylphenol, identified as the main component, has a characteristic unpleasant odor. Sweet and mild odor was attributed to lactones. 2-Acetylthiazole had a cereal-like odor. It was concluded that lactones and 2-acetylthiazole are major contributors to fresh rice bran aroma.

Milled Rice

Removal of the bran layers (pericarp, tegmen, nucellus, and aleurone) along with polish (subaleurone), germ (embryo), and a small part of the endosperm results in milled (white) rice composed entirely of endosperm, with a starch content of approximately 78% (14% moisture) or 90% (dry weight) (Table 1). Waxy rice

starch has an apparent amylose content of 0.8–1.3%, whereas the amylose content of nonwaxy rice starch ranges from 8 to 37%. Starch concentration increases from the surface to the core of milled rice. Protein is the second most abundant constituent of milled rice, with content ranging from 4 to 11%. Protein concentration is highest on the surface of milled rice and decreases toward the center of the kernel. The albumin-globulin-prolamin-glutelin ratio for a milled U.S. long-grain rice was 8:10:12:70 (Padhye and Salunke, 1979). Ratios for japonica rice cultivated in Korea were 1.8–2.4:10.8–15.1:1.8-5.2:79.1–85.5 (Kim et al, 1997). A basmati rice had a ratio of 5.9:13.8:5.8:74.5 (Steenson and Sathe, 1995). Six varieties of Indonesian rice showed ratios of 8:12–14:1–2:77–79 (Damardjati et al, 1985). The ratios obtained for rice are unique among cereal proteins in that the glutelin fraction is predominant, whereas other cereals, except oats, have prolamins as the major fraction (Payne and Rhodes, 1982). This results in the lysine content of rice being higher than in other cereals because prolamins are lysine-poor.

Crude fiber and lipid content in milled rice are low, ranging from 0.2 to 0.5% and 0.3 to 0.5%, respectively (Table 1). Phosphorus and potassium are the most abundant minerals in milled rice (Table 2). Niacin, inositol, and choline are the three predominant vitamins (Table 3). The distribution of fiber, lipid, and vitamins in the milled grain follows that of the protein.

A large number of volatile compounds have been observed in uncooked and cooked milled rice. In all, 100 volatile constituents, including 13 hydrocarbons, 13 alcohols, 16 aldehydes, 14 ketones, 14 acids, 8 esters, 5 phenols, 3 pyridines, and 6 pyrazines, were identified by analysis of steam distillate of cooked rice by glass capillary gas chromatography (GC) and mass spectrometry (MS) (Yajima et al, 1978). The majority of the compounds were lipid oxidation products and, based on their high odor thresholds, would contribute minimally to the aroma of fresh rice. With deterioration during storage, they would contribute to what is referred to as "old" or "stale" rice aroma. Grimm et al (2001) reported 138 compounds observed in the headspace of a worldwide collection of uncooked milled rice varieties. The headspace was collected by selective phase microextraction and analyzed by GC/MS. The GC profile was dominated by lipid oxidation products, including straight-chain aldehydes from pentanal to dodecanal, the alcohols from pentanol to dodecanol, and short-chain fatty acids from acetic to decanoic acid. There was also a series of unsaturated aldehydes ranging from (*E*)-2-heptenal to (*E*)-2-decenal.

Volatile sulfur compounds evolved from cooked rice were hydrogen sulfide, methyl mercaptan, dimethyl sulfide, *n*-butyl mercaptan (butane-1-thiol), and dimethyl disulfide (Sato et al, 1976; Tsuzuki et al, 1978).

Buttery et al (1988) identified 64 major volatile compounds in rice and concluded from odor threshold data that the probable major contributors to rice aroma in the grain examined were 2-acetyl-1-pyrroline, (*E,E*)-2,4-decadienal, nonanal, hexanal, (*E*)-2-nonenal, octanal, decanal, 4-vinyl-guaiacol, and 4-vinylphenol. 2-Acetyl-1-pyrroline, which has a characteristic popcorn aroma, is found at concentrations ranging from 1 to 10 ppb in nonaromatic rice and in excess of 2 ppm in scented rice (Buttery et al, 1988). It is largely responsible for the aroma of the aromatic basmati- and jasmine-type varieties. In a comparative study of nonfragrant and fragrant rice, Widjaja et al (1996) identified (*E*)-2-decenal, (*E,E*)-2,4-nonadienal, and (*E,E*)-2,4-decadienal as having a "waxy" aroma. These three lipid oxidation products also are found in waxy rice and contribute to its aroma (Grimm et al, 2000).

Brown Rice

Brown rice, the grain with just the hull removed, has a higher content of all constituents, except starch, compared with milled rice (Tables 1–3). The concentration of the vitamins is typically 2–10 times higher in brown rice than in milled rice. Mineral and fiber contents are two to three times higher in the brown rice, and lipid content is approximately five times higher.

The brown rice protein content of 17,587 cultivars in the world collection at the International Rice Research Institute ranged from 4.3 to 18.2%, with a mean of 9.5% (Gomez, 1979). Nonprotein nitrogen, mainly free amino acids, accounts for ~3% of the total nitrogen of brown rice, with the highest level in the germ (Juliano, 1972). The major free amino acids are asparagine + aspartic acid, glutamic acid + glutamine, alanine, serine, proline, γ-aminobutyric acid, glycine, arginine, threonine + valine, and tryptophan (Tamura and Kenmochi, 1963; Fujimaki et al, 1975).

Calculation of nutrient distribution in the milling fractions of brown rice confirms the uneven distribution of nutrients in the various tissues of the rice caryopsis (Resurreccion et al, 1979). More nonstarch constituents are removed during milling, with fiber showing the most drastic drop (Table 6), followed by the other nutrients, except protein and zinc. A less clear-cut effect of milling is noted for ash and its component phosphorus, potassium, and iron. In 12%-milled brown rice, protein distribution is 22% in bran-polish and 78% in milled rice (Table 6). Protein distribution becomes more even as protein content increases in the grain (Cagampang et al, 1966; Juliano et al, 1973; Resurreccion et al, 1979). The highest protein fraction in the high-protein grain is the polish, rather than the bran (18.8 versus 14.0% protein) (Resurreccion et al, 1979). Free amino acid distribution is calculated as 30% in the bran-polish, 53% in the embryo, and 17% in milled rice (Tamura and Kenmochi, 1963).

TABLE 6
Nutrient Distribution in Milling Fractions of IR32 Rice Caryopsis[a]

		Distribution (% of nutrient in brown rice)				
				Endosperm (milled rice)[b]		
Nutrient	Content in Brown Rice (% at 14% moisture)	Bran	Polish	Sub-aleurone	Middle	Core
Weight (%) of brown rice	100	0–6	6–12	12–20	20–30	30–100
Protein (N × 5.95), %	7.2	11	11	13	14	51
Starch, %	66.4	1	4	7	11	77
Amylose, %	26.5	1	3	8	10	78
Crude fiber, %	0.7	70	19	1	1	9
Neutral detergent fiber, %	2.1	71	19	1	2	7
Crude fat, %	2.9	51	32	10	1	6
Crude ash, %	0.78	42	26	10	5	17
Total P, %	0.15	38	25	11	4	22
Phytin, P, %	0.1	47	33	16	1	3
Total K, %	0.19	37	22	9	5	27
Total Mg, %	0.08	50	34	11	1	4
Total Fe, μg/g	12	47	24	10	3	16
Total Zn, μg/g	27	31	14	7	6	41

[a] Reprinted, with permission, from Resurreccion et al (1979).
[b] Distribution (% of nutrient in brown rice).

The distribution of brown rice pentosans by calculation is 43% in bran, 8% in germ, 7% in polish, and 42% in milled rice (Leonzio, 1967). The corresponding cellulose distribution is 62% in bran, 4% in germ, 7% in polish, and 27% in milled rice (Leonzio, 1967). Lignin distribution in brown rice is calculated as 63% in bran, 8% in polish, 8% in germ, and 21% in milled rice (Leonzio, 1966).

About 80% of the nonstarch lipids of brown rice is in the bran and polish, and about one-third of this is in the embryo (Juliano, 1972). Choudhury and Juliano (1980a) calculated nonstarch lipids distribution in IR42 brown rice as 41% in bran, 18% in embryo, 15% in polish, and 26% in milled rice (12% in the subaleurone layer). Starch lipids are mainly in the endosperm, because they are associated with amylose (Choudhury and Juliano, 1980b).

The ash distribution in brown rice is calculated as 51% in bran, 10% in germ, 11% in polish, and 28% in milled rice (Leonzio, 1967). Iron, phosphorus, and potassium show a distribution similar to that of total ash (Resurreccion et al, 1979) (Table 6). However, some minerals show a relatively more even distribution in the grain. For example, calculation has shown that milled rice retains 63% of the sodium and 74% of the calcium content of brown rice.

By calculation, 65% of the thiamin of brown rice is in the bran (58% in the embryo), 13% in polish, and the rest (22%) in milled rice. Corresponding values for riboflavin are 39% in bran (24% in embryo), 8% in polish, and 53% in milled rice. The distribution of niacin is 54% in bran (18% in embryo), 13% in polish, and 33% in milled rice. Milling results in the loss to human consumption of ~76% of the thiamin, 57% of the riboflavin, and 64% of the niacin of brown rice (Kik and Williams, 1945).

GC/olfactometry of cooked brown rice samples revealed 41 odor-active compounds, of which the following 12 compounds had high flavor dilution factors (low odor thresholds): 2-acetyl-1-pyrroline (popcorn-like), 2-isobutyl-3-methoxypyrazine (earthy, green bell pepper), (*E,E*)-nona-2,4-dienal (fatty), 4,5-epoxy-(*E*)-dec-2-enal (metallic), unknown (spicy), bis-(2-methyl-3-furyl)-disulfide (meaty), 3-hydroxy-4,5-dimethyl-3(2H)-furanone (seasoning-like), 4-vinyl-2-methoxyphenol (spicy, clove-like), 2-amino acetophenone (medicinal, phenolic), 4-vinylphenol (phenolic), phenyacetic acid (honey-like), and vanillin (vanilla-like) (Jezussek et al, 2002). 2-Amino acetophenone exhibited the highest flavor dilution factor. Differences in flavor dilution factors for some compounds were observed among varieties.

FACTORS AFFECTING COMPOSITION

Management and Cultural Practices

Management and cultural practices have a major influence on the protein content of the rice grain. Protein content was observed to be higher when wider plant spacing was used, and this was attributed to more nitrogen being available per plant (De Datta et al, 1972). However, a later study showed that spacings had no significant effect on protein content (Nair, 1975). Protein content was generally higher in hills bordering an unplanted alley or in hills adjacent to missing hills (de Datta et al, 1972). Protein content was shown to decrease with increasing number of seedlings per hill, irrespective of spacing (Chandra et al, 1990).

Nitrogen, phosphorus, and potassium fertilizer treatments are reported to increase concentrations of nitrogen (protein), phosphorus, sulfur, manganese, magnesium, calcium, and copper (Sturgis and Reed, 1937; Patrick et al, 1974; Tsuzuki et al, 1979; Chen et al, 1997; Marr et al, 1999; Haefele et al, 2002). Nitrogen top-dressing during the period from the reproductive to the early ripening stage of plant development plays a major role in determining the protein content of the rice grain. Top-dressing with nitrogen at flowering resulted in a 30–60% increase in head rice protein content (Perez et al, 1996). Islam et al (1996) also observed increased crude protein with nitrogen top-dressing, which was considerably greater in the indica than in the japonica variety examined. The top-dressed ^{15}N partitioned most effectively to the glutelin fraction when it was applied at the heading stage. On the other hand, ^{15}N applied at heading plus 20 days contributed more to increased prolamin. Souza et al (1993) found that glutelin increased and prolamin decreased with increases in protein content resulting from supplementary nitrogen. However, in contrast, Takebe et al (1996) found that the proportions of individual proteins (albumin + globulin, glutelin, and prolamin) in the total protein remained almost constant as protein increased with increasing amounts of applied nitrogen. The total amount of free amino acids has been observed to decrease (Araki et al, 1999) and increase (Souza et al, 1999) with nitrogen application.

A decrease in amylose content has been observed concurrent with an increase in protein content with nitrogen application or uptake (Prakash et al, 2002). Zinc (Chen et al, 1997) and potassium (Vil'gel', 1986) applications, conversely, increased amylose content of rice. Applied magnesium also increased grain amylose and protein content, with the response to applied magnesium being negatively correlated with soil magnesium levels and positively correlated with soil potassium-magnesium ratio (Lin et al, 1990).

For brown rice, nitrogen application decreased the riboflavin content (from 1.41–1.43 μg/g to 1.16–1.27 μg/g) and increased the nicotinic acid content (from 54.7–55.4 μg/g to 58.3–62.1 μg/g), but it had no effect on milled rice content (Taira et al, 1976). Thiamin and folic acid were not affected by nitrogen fertilization.

The composition of organically and conventionally grown rice was compared over three years at 13 different locations (Nakagawa et al, 2000). At each location, organically and conventionally managed paddy were grown in adjacent fields. The organically grown rice had a higher magnesium-(potassium + nitrogen) ratio and zinc content and lower nitrogen, potassium, and calcium content. All except calcium content could be explained by lower nitrogen application in organic farming systems. Tamaki et al (1995) determined the mineral content of rice grown organically over 16 years. The mineral content of rice one year after the start of organic farming practices was similar to that of rice grown conventionally. Nitrogen, phosphorus, and potassium content decreased with the duration of organic farming, while magnesium content gradually increased. These changes were greatest in the first five years of organic farming. Of the free amino acids, aspartic acid, glutamic acid, glutamine, and asparagine were significantly higher in milled rice grains from organically grown rice than in those from conventionally grown rice (Wang et al, 1998). In contrast, the concentrations of hydrolyzed amino acids tended to be higher in milled rice obtained from conventional than from organic practices.

Protein and amylose content were observed to decrease with delay in harvest time irrespective of cultivation method (organic or traditional) (Asano et al, 2000). The composition of the protein, however, was unaffected by harvest time.

CHAPTER 5

STARCH

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Starch is one of the most important agricultural products for the world's population. Currently, 2,050 million tonnes of starch each year are produced from cereals (Khush, 1997), and rice contributes significantly to that figure (Burrell, 2003). Food use accounts for most of the starch, insofar as it can represent up to 80% of daily calorific intake in some cultures (Burrell, 2003). Starch is also used in many non-food applications, including adhesives, coatings, pharmaceuticals, fillers, viscosity modifiers, and a host of others. Each application requires particular properties of the starch, and the properties of starch are largely defined by the macro-, micro-, and ultrastructure of the starch.

Starch granules constitute approximately 90% of the dry weight of a milled rice grain. Starch is polyglucose, and the two types of glucose polymers that constitute starch are amylose and amylopectin. Starch determines the physical and cooking properties of rice grains, or at least contributes to them through interactions with other components of the rice endosperm (proteins, lipids, water) or through interactions with other ingredients used to process the rice. The ratio of amylose and amylopectin in the starch, the solubility of each, and the structure of each fraction all contribute to the performance of the rice grain. The synthesis and structure of starch has long been a mystery, but because of the evolution of technology over the past decade, knowledge of starch synthesis and structure has grown exponentially. This deeper understanding makes it a real possibility that starch structure can one day be manipulated to target starches for particular markets and applications and to assist in identifying novel and potential applications.

THE STARCH GRANULE

Starch granules are made from two polymers of glucose: amylose and amylopectin. Amylopectin molecules are highly branched, are of high molecular weight, and constitute the skeleton of the starch granule (Kossmann and Lloyd, 2000). Amylose molecules are essentially linear, but have some branching, and exist in starch granules along with amylopectin. Amylopectin is essential for the synthesis of a starch granule, but amylose is not. Amylose has received far more attention in rice research than has amylopectin, even though it accounts for much less of the starch, since it is considered to be a key indicator of cooking quality.

Starch granules that lack amylose are called either waxy, because of their mutation at the waxy locus (Mizuno et al, 1993), or glutinous, because of their opaque appearance. The structure of the starch granules differs among the cereals, and starch granules in cereal endosperms differ from those in tubers and roots (Martin and Smith, 1995). Starch granules in rice differ from starch granules in wheat, corn, and barley. Those in corn range in shape from round to angular (Whistler and BeMiller, 1997). Wheat grains contain two populations of granules, one about 40 μm in diameter, and the other ranging from 5 to 16 μm (Whistler and BeMiller, 1997). Starch granules of rice are small, about 3-5 μm each, and each granule is angular. The granules are compound (Fig. 1). Each compound starch granule is polyhedral and contains many (at least 16) individual granules (Fig. 1). The only other cereal with compound starch granules in the endosperm is oats. The formation of compound granules has received little attention, perhaps because the compound structure itself is not a recognized source of variation affecting the end use of the grain.

Starch granules contain some proteins and some lipids. The proteins are mainly enzymes remaining from the synthesis of starch (Martin and Smith, 1995) and are mostly accounted for by granule-bound starch synthase (GBSS) (Baldwin, 2001). By contrast, in the endosperm, the protein is storage protein in bodies outside the starch granules (Chrastil, 1990).

Lipids complexed with starch molecules occur in starch granules. During synthesis of the starch molecules, complexes form between amylose and lipids as a result of the lipid molecule lodging in the cavity of the amylose helix (Morrison et al, 1993). There is also some suggestion of a complex forming between lipids and long chains on the amylopectin molecule. The presence of the lipids can substantially affect the cooking, processing, and sensory properties of the starch, and the structure of the starch can affect the types of lipids present (Ozcan and Jackson, 2002).

Fig. 1. Scanning electron micrograph of a fractured plane of a rice grain showing two compound amyloplasts broken open, clearly revealing at least 16 single granules (see arrows) inside the compound structure.

Macrostructure of the Starch Granule

CRYSTALLINE REGIONS

Starch granules contain crystalline and amorphous areas and are therefore described as semicrystalline. The radial and ordered arrangement of the starch molecules confers the semicrystallinity. Starch granules are birefringent when viewed in polarized light, showing a cross, commonly known as the maltese cross (Whistler and BeMiller, 1997). The birefringence is due to the high degree of molecular order of the starch granules but does not actually define crystallinity. The technique of X-ray diffraction provides information about crystallinity and is used to show the structure of the crystallites, changes to crystallite structure after different treatments, and relative amounts of crystalline and amorphous regions. Figure 2 (top) shows a diagram of the molecular packing in the A and B types of polymorphs. X-ray diffraction of intact granules of cereal starch shows that starch granules from cereals give the A pattern of crystallinity (Whistler and BeMiller, 1997). The A pattern indicates that the crystalline regions contain parallel double helices

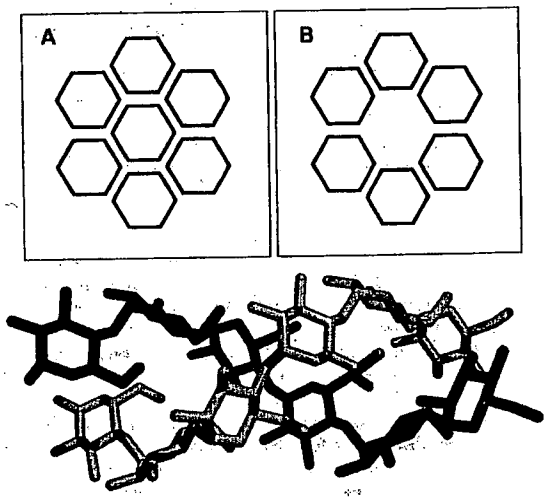

Fig. 2. Top, simple diagram of the A and B polymorphs of starch. The A polymorph is common in cereals and contains less interhelical water than the B polymorph, which is common in tubers. The hexagons represent the double helices depicted in the bottom diagram, each of which has six residues/turns Bottom, three-dimensional diagram of a double helix. (Courtesy Alfred French and Glenn Johnson, USDA-ARS Southern Regional Research Center)

separated by interhelical water (Gidley, 1987). The A polymorph contains four molecules of water per unit cell, and the B polymorph, commonly found in starch from tubers, holds 36 molecules of interhelical water per unit cell (Baker et al, 2001). Figure 2 (bottom) depicts a double helix.

Microscopic studies suggest that the crystalline and amorphous regions are arranged in blocklets that appear as protrusions on the surface of the granule when viewed with scanning electron microscopy and atomic force microscopy (AFM) (Gallant et al, 1997; Dang and Copeland, 2003). Gallant et al (1997) propose that the different layers of the starch granule contain different-sized blocklets; larger blocklets occur in the crystalline shells, and smaller blocklets occur in the semicrystalline shells. The studies so far suggest that the size of the blocklets varies also with the botanical origin of the starch (Gallant et al, 1997). The size of blocklets in rice starch from several varieties has been estimated by AFM to be about 100 nm (Dang and Copeland, 2003). Blocklets in rice starch are proposed to contain about 280 amylopectin side-chain clusters alternating with amorphous regions estimated to be about 4 nm long (Dang and Copeland, 2003).

Amylopectin clusters arise from the crystallization of side chains into mature amylopectin, removing those chains from further synthetic processes. Crystallization generally occurs by the formation of double helices between adjacent A chains of amylopectin. A single turn of a single chain in a double helix requires a maltooligosaccharide to be six units long. Studies show that chains as short as six glucose units can cocrystallize, but optimally chains need to be about 10–12 glucose units to crystallize (Gidley and Bulpin, 1987; Imberty et al, 1991). Double helices are stabilized by hydrogen bonding and van der Waals forces (Imberty et al, 1991). A double helix can form between the outer chain of one amylopectin molecule and the outer adjacent chain of a neighboring molecule (Hizukuri, 1986). Groups of A chains are clustered in the same plane on different molecules, and the planar clustering allows double helices to form within and between molecules on that plane, leading to arrays of crystallinity. Studies show that, within an amylopectin molecule, clusters are approximately 9 nm long, 120–400 nm wide, and separated by amorphous areas about 4 nm long (Watanabe and French, 1980; Gallant et al, 1997; Knight et al, 1998).

AMORPHOUS REGIONS

Two types of amorphous areas occur in starch granules: channels extending from the outer surface to the center of the granule; and regions that alternate with crystalline regions (Gallant et al, 1997). The amorphous regions that alternate with crystalline regions probably account for most of the amorphous area of the starch granule. These areas are rich in amylopectin branch points (Robin et al, 1974) and are also likely to contain the amylose (Jacobs and Delcour, 1998). A study using cross-linking agents showed amylose cross-linked with amylopectin, which led the authors to suggest that amylose chains extend from the amorphous regions into the crystalline regions of the amylopectin (Jane et al, 1992). More recently, gold-labeled enzymes were used to label amylose and amylopectin; that work indicated that discrete bands of amylose alternate with discrete bands of amylopectin, thereby placing amylose in the amorphous region (Atkin et al, 1999). Another study, on the synthesis of amylose, also suggests that, while amylose is being extended, the growing polymer is extruded into the spaces in the amorphous areas (Vandewal et al, 1998).

Surface pores have been observed in starch granules from rice, wheat, and potatoes (Baldwin et al, 1994), and both pores and channels have been observed in starch from wheat, rye, barley, millet, corn, and sorghum (Gallant et al, 1997). The channels are thought to extend from the surface pores, creating a serpentine pathway into the granule along the junction zones of the blocklets (Gallant et al, 1997). Channels are thought to facilitate the exchange of water to and from the starch granule (Baldwin et al, 1994) and to provide exit points for amylose as it leaches during gelatinization of the starch (Gallant et al, 1997).

The ratio of amylose and amylopectin in the starch granule has been shown to alter the relative sizes of the crystalline and amorphous areas (Jenkins and Donald, 1995). The length of the glucan chains, the structure of the individual starch molecules, and the packing of the individual molecules all contribute to the radial and ordered arrangement of both the crystalline and amorphous regions (Gidley, 1987; Gidley and Bulpin, 1987).

Microstructure of the Components

AMYLOPECTIN

The structure of starch differs with botanical origin (Smith et al, 1997). Mutants carrying a lesion in GBSSI—i.e., waxy mutants—accumulate starch granules morphologically similar to those in wild-type endosperms, except that they lack amylose (Yun and Matheson, 1993). Amylopectin molecules therefore are required for the skeleton of the starch granules, and amylose perhaps fills the spaces.

The structure of an amylopectin molecule has never been visualized microscopically, but interpretations have been drawn from studies using enzymatic digestion of the molecule, gel permeation and size-exclusion chromatography, electrophoresis, absorption spectroscopy, or reducing-end chemistry. Two classes of enzymes have been useful in investigations of the structure of amylopectin. One of these classes contains the debranching enzymes isoamylase and pullulanase. These enzymes digest α-(1-6) linkages only, which essentially decomposes the amylopectin molecule into its constituent chains, allowing the chain-length distribution of amylopectin to be determined. The second class of enzymes, which includes β-amylase, digests α-(1-4) linkages only, liberating maltose. Sequential digestion with such enzymes, coupled with measurements of the glucose released as maltose and of the reducing power of the product, has led to interpretations of the length of the different chains and a proposal of amylopectin structure, the cluster model, which is the currently accepted one (Kainuma and French, 1972; Robin et al, 1974). The central chain, carrying the reducing end, is termed the C chain; B chains subtend the C chain, and A chains and other B chains subtend the B chains. The A chains are connected to other chains through α-(1-6) linkages but are not branched themselves. There are many more A chains than B chains, and the A chains are arranged in ordered clusters and define the areas of crystallinity. The B chains can be divided further into four populations of chains. B4 chains span four clusters of A chains; B3 chains span three clusters; B2 span two clusters; and B1 chains span only one cluster (Hizukuri, 1986). The C chains range in size from 10 to 130 glucose units, with most being around 40 glucose units (Hanashiro et al, 2002).

The architecture of the amylopectin molecule is determined by the length, frequency, and placement of its constituent chains. Amylopectin molecules in rice fall into three broad groups, with average degrees of polymerization (DP) of 700–2,100

(small), 4,400–8,400 (medium), and 13,400–26,500 (large) (Takeda et al, 2003). The chain-length distribution of amylopectin from B-type starches is longer than that from A-type starches (Hizukuri et al, 1983), and within the polymorph classes, the chain-length distribution differs among species (Hizukuri et al, 1983; Reddy et al, 1993; Ong et al, 1994; Hanashiro et al, 1996, 2002; Hanashiro and Takeda, 1998; Takeda et al, 2003), and between indica and japonica rices (Hizukuri et al, 1989; Umemoto et al, 2002). The chain-length distribution of starches has become much simpler to assay since the development of capillary electrophoresis techniques (Oshea et al, 1998). Chain-length distribution by capillary electrophoresis has been applied to rice starch and used to compare varieties, describing more fully the difference between the chain-length distributions of indica and japonica rices (Nakamura et al, 2002), and to determine the function of different enzymes of starch synthesis (Nakamura et al, 2002; Umemoto et al, 2002).

AMYLOSE

Amylose is the other polymer of glucose in starch granules. Amylose chains, like amylopectin, are glucose polymers, but unlike amylopectin, are linked mainly through α-(1-4) linkages. Amylose chains are extremely long relative to those of amylopectin, about 1,000 DP (Takeda and Hizukuri, 1987). Experimental evidence confirms that some branching occurs on the amylose chains. Incomplete hydrolysis of potato amylose, using β-amylase, which completely digests linear α-(1-4)-linked chains, indicated that amylose carries branches (Hizukuri et al, 1981). The researchers then coupled α-amylase with the enzyme pullulanase, which hydrolyzes α-(1-6) linkages, and the two enzymes completely hydrolyzed the amylose (Hizukuri et al, 1981), again suggesting branching. Branching has recently been essentially confirmed microscopically in amylose from pea (Gunning et al, 2002). In the rice variety Sasanishiki, Takeda and Hizukuri (1987) found that each amylose molecule was subtended by 8–11 branches and that the average length of those chains was 115 DP. Furthermore, the branching structure of amylose differs between and within species (Takeda and Hizukuri, 1987).

It is not surprising that the branching structure of amylose differs among varieties within a species. Different varieties of rice carry different alleles of GBSSI (Ayres et al, 1997; Bergman et al, 2001), possibly different alleles of starch branching enzyme (SBE) (Bao et al, 2002), and possibly different factors affecting the expression of GBSS or SBE (Larkin and Park, 1999; Cai et al, 2002). Therefore, different varieties of rice are likely to differ in the frequency, length, and placement of branches on amylose backbones, and therefore the structure of amylose is likely to differ among varieties.

When starch is heated in water, amylose leaches from the granules and constitutes the hot-water-soluble component (Tsai et al, 1997). Presuming that the amylose is free to leach, the structure of the amylose could cause it to become entangled in the gelatinizing and swelling starch granule, thus causing it to be unable to leach. In different varieties of rice, amylose differs in the amount that is soluble in hot water (Bhattacharya et al, 1978; Ramesh et al, 1999; Mizukami et al, 1999). Figure 3 shows the distribution of hot-water-soluble amylose by gel permeation chromatography from three different varieties of rice, all of 25% amylose. Molecules with weights between 5.10^3 and 5.10^5 (~30–3,000 DP) elute in the separating phase of the column. Clearly, the total amylose in a rice grain does not reflect the amount of hot-water-soluble amylose. In that case, the structure of amylose in each individual starch granule could differ.

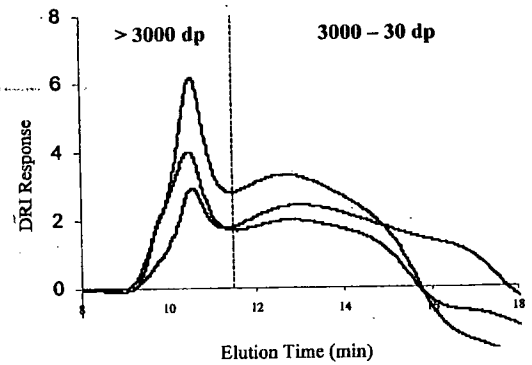

Fig. 3. Chromatogram from a gel-permeation column of solubilized starch from three rice varieties, each with 25% amylose. Polymers greater than about 3,000 degrees of polymerization (DP) elute in the void volume, and those less than 3,000 DP elute in the separating phase of the column. Amylose elutes in the separating phase. Each variety shows a different amount of soluble amylose and a different molecular weight of the amylose. DRI = differential refractive index.

There may be little variation in the molecular weight distribution of amylopectin molecules for any given rice (Takeda et al, 2003), but there certainly is a broad molecular weight distribution of amylose chains. Figure 3 shows the molecular weight distribution of hot-water-soluble amylose from three varieties of rice by gel permeation chromatography. It is clear from Figure 3 that a significant amount of material with molecular weight greater than 5.10^5 (~3,000 DP) elutes in the void volume, and a significant amount of material elutes in the separating phase of the column, which ranges in molecular weight distribution from about 30 to 3,000 DP. Such a range in the molecular weight of amylose from any given rice suggests that the length of the amylose chains is not controlled by GBSSI. Consistent with this is the idea that amylose is synthesized in accordance with the space available in the starch granule, and as space becomes progressively limiting, the size of amylose progressively decreases (Vandewal et al, 1998).

Starch granules have evolved to store a large amount of glucose in a condensed, relatively dehydrated form, to provide energy to nourish the next generation during germination and during the early stages of growth. However, the starch in different genera, species within a genus, and cultivars within a species differ in macro-, micro-, and ultrastructure, and consequently these variations cause different functional properties. Because starch accounts for at least 90% of the dry weight of the rice grain, the functional properties of starch, alone and interactively, are of extreme interest to any customer or consumer of rice.

FUNCTIONAL PROPERTIES OF STARCH

During cooking, several stages occur to transform a raw rice grain into a cooked grain of pleasing textural attributes. These include glass transition, gelatinization, swelling, pasting and leaching of amylose, and retrogradation. Glass transition and

gelatinization are characteristics of starch as a semicrystalline and glassy polymer system (Biliaderis et al, 1985; Slade and Levine, 1988, 1989; Waigh et al, 2000). Glass transition occurs before gelatinization, and therefore determines the gelatinization temperature (Slade and Levine, 1988). Water is a plasticizer that depresses the temperature at which both glass transition and gelatinization occur, and temperature affects the plasticizing capacity of water (Slade and Levine, 1988). The addition of water and heat begins the process of cooking, and thus glass transition and gelatinization affect many of the rheological properties of the rice as whole grains and as an ingredient in other applications.

Following gelatinization, the granule can swell to many times its original volume, and in the absence of shear, it maintains its structure (Parker and Ring, 2001). During swelling, the amylose hydrates and leaches from the granule. The leached amylose is then able to interact with other amylose molecules and form a gel embedding the swollen amylopectin molecules and hydrated proteins, thus forming a cooked rice grain or gel with a particular collection of textural attributes.

Glass Transition

Before gelatinization of starch can occur, the glassy (amorphous) regions of the starch must become soft and rubbery (Biliaderis et al, 1986; Slade and Levine, 1987). This process is called glass transition (Biliaderis et al, 1985, 1986; Slade and Levine, 1988). Water depresses the glass transition temperature (T_g), and so at higher moisture contents, glass transition occurs at lower temperatures (Biliaderis et al, 1986; Slade and Levine, 1988, 1989; Matveev et al, 2000). In the conditions required to cook rice, the rice is generally in excess water, so glass transition is always depressed. When rice is used as an ingredient in a process, the moisture content can be used to manipulate the glass transition and thereby the processing properties of foods (Slade and Levine, 1988, 1989). For a synthetic polymer, glass transition occurs when a glassy region is at a certain moisture content and temperature that allows it to become rubbery, and when those conditions are removed, the effects of glass transition are reversed. In terms of starch, though, the glassy, or amorphous, areas contain the branch points of the amylopectin, many of which subtend chains that traverse crystalline clusters on either side of the amorphous regions (Biliaderis et al, 1986; Slade and Levine, 1987), so reversing glass transition may not necessarily reverse the secondary effect of rubbery amorphous regions.

Gelatinization

Gelatinization is the non-equilibrium melting of the crystalline regions (Slade and Levine, 1988), and requires presoftening of the amorphous regions (glass transition). Gelatinization temperature is an important property of rice, or of any grain, because it correlates strongly with the cooking time and the texture of the cooked product (Maningat and Juliano, 1978). Gelatinization temperature is influenced by the extent of crystallinity (Slade and Levine, 1988), the proportion of short chains in amylopectin (Qi et al, 2003)—which is probably another way of expressing the extent of crystallinity, and the proportion of long chains in amylopectin (Umemoto et al, 2002).

Swelling and Pasting

Once starch gelatinizes, the starch granules begin to swell and, in the absence of shear, can swell to many times their initial volume while maintaining their integrity (Parker and Ring, 2001). The swelling is accompanied by leaching of amylose molecules into the continuous phase (Bhattacharya et al, 1972, 1978; Chinnaswamy and Bhattacharya, 1986; Jacobs and Delcour, 1998; Nguyen et al, 1998; Rani and Bhattacharya, 1989; Mizukami et al, 1999; Ramesh et al, 1999; Fitzgerald et al, 2003). In many studies, the amount of amylose that becomes soluble in hot water has been reported to indicate and influence cooked texture more than the effect of the total amount of amylose (Chinnaswamy and Bhattacharya, 1986; Sowbhagya et al, 1987; Ramesh et al, 1999). However, in an earlier study, the importance of the insoluble amylose was emphasized (Bhattacharya et al, 1978). The confusion probably arose because several things influence the solubility of amylose. Solubility of the amylose differs among varieties, and the extent of solubility could be due to the structure of the amylose, entanglement with gelatinized amylopectin (and therefore the structure of amylopectin), or complexes between amylose and lipids.

Retrogradation

Gelatinized starch contains no crystalline regions, but under certain conditions of storage and temperature, the molecules in a starch gel can reassociate into an ordered structure (Baik et al, 1997). This process is called retrogradation, and in terms of food technology, refers to the hardening of cooked rice or a starch gel upon storage. The deterioration of the textural characteristics of cooked rice is due, largely, to retrogradation of the starch (Yao and Ding, 2002).

Retrogradation describes the rapid recrystallization of amylose and the slow recrystallization of amylopectin (Slade and Levine, 1987). The degree of retrogradation and the nature of the newly formed crystals can depend on the time and temperature of storage, the source of the starch, and the presence of other molecules in the system (Slade and Levine, 1987; Baik et al, 1997).

Recrystallization of amylose is essentially the rapid formation of double helices in parts of the amylose chains followed by aggregation of those helices (Gidley, 1989). The first stage of retrogradation depends on the amylose content of the rice (Baik et al, 1997) and the amount of amylose that is free, rather than complexed with lipids (Yao et al, 2002). Further, hot-water-soluble components of rice starch with high molecular weight promote retrogradation more than lower-molecular-weight polymers (Tsai and Lii, 2000), suggesting that the molecular weight distribution of the amylose contributes significantly to the first phase of retrogradation. Retrogradation due to amylose is not reversible at temperatures less than 100°C (Miles et al, 1985) because amylose crystals melt only above 100°C.

Recrystallization of short amylopectin chains constitutes the second process of retrogradation (Baik et al, 1997). Several studies suggest that the fine structure or the chain-length distribution of amylopectin contributes to differences in the degree of retrogradation by amylopectin, in particular, the proportion of short A chains in the amylopectin (Lu et al, 1997; Fredriksson et al, 1998; Silverio et al, 2000; Tsai and Lii, 2000; Yao et al, 2002). Further, Yao et al (2002) suggest that the interaction of amylose with amylopectin increases the rate of amylopectin retrogradation. Retrogradation due to amylopectin is reversible if the retrograded gel is exposed to a

temperature greater than the gelatinization temperature of crystalline amylopectin (Lu et al, 1997).

Clearly, the functional properties of rice differ greatly among different varieties of rice. These functional properties include the extent of crystallinity, glass transition, gelatinization temperature, the ability of granules to swell following gelatinization, the solubility of the amylose, and the rate and degree of retrogradation.

These variations must be due, at least in part, to different macro-, micro-, and molecular structures of the starch and must arise from different synthetic processes or from different regulatory mechanisms affecting similar processes. Understanding the processes of starch synthesis in different species, genera, and cultivars provides opportunities to manipulate starch synthesis to generate novel starch structures.

THE ENZYMES OF STARCH SYNTHESIS

During the day, the vegetative parts of the plant produce sugars, mainly sucrose and other nonreducing sugars, from photosynthesis. These sugars are transported from their source, the bundle sheath cells of the leaves, to the various sink organs within the plant. During the generative phase of plant growth (i.e., during grain development), the developing grains exert the strongest sink demand in the plant. Sucrose produced from photosynthesis is then transported in the phloem to the developing grains.

Starch accumulates in the rice grain during the grain-filling period. Its deposition involves a suite of enzymes and several processes. The first process is the acquisition of the precursors, which are small sugars, including sucrose and small polymers of glucose. That process relates more to plant physiology than to starch chemistry and is not dealt with here. The second process is the synthesis of the amylose and the amylopectin. A rich body of literature, which is constantly being updated, discusses the synthetic processes (Martin and Smith, 1995; Sinclair et al, 1995; Ball et al, 1996, 1998; Morell et al, 1997; Smith et al, 1997; Isshiki et al, 1998; Vandewal et al, 1998; Denyer et al, 1999a,b, 2001; Kossmann and Lloyd, 2000; Myers et al, 2000). The third process is the organization of the starch polymers into crystalline, semicrystalline, and amorphous regions and the organization of amylose into its location within the starch granules. The fourth process, unique to rice and oats, is the organization of individual starch granules into compound starch granules. The third and fourth processes are described above.

Adenosine 5′-Diphosphatase Glucose Pyrophosphorylase

Starch synthesis in rice becomes most active about 10 days after pollination; after the endosperm sac has expanded and cellularized (Evers and Millar, 2002). In those initial 10 days, the endosperms acquire the nutrients and precursors they require for the synthesis and deposition of starch (and proteins and lipids) (Jenner et al, 1993; Jenner, 1994; Bhullar and Jenner, 1986). Sucrose is the main form of carbon delivered to the developing endosperm, but the sucrose must be metabolized to glucose-1-phosphate (G1P), most likely by the agency of sucrose synthase and uridine 5′-diphosphatase (UDP) glucose pyrophosphylase (Kossmann and Lloyd, 2000; Emes et al, 2003) to allow the glucose to participate in the pathway of starch synthesis. Four classes of enzymes contribute to the synthesis of starch from adenosine 5′-diphosphatase (ADP) glucose: ADP glucose pyrophosphorylase (AGPase), the starch synthases, SBEs, and starch-debranching enzymes (Kossmann and Lloyd, 2000). Figure 4 shows a diagram of the pathways of starch synthesis.

The first committed step in the synthesis of starch is the conversion of G1P and adenosine 5′-triphosphatase (ATP) to ADP-glucose (ADPG) by the action of AGPase (EC 2.7.7.23), producing pyrophosphate (Martin and Smith, 1995). It was once believed that the main activity of AGPase occurred in the amyloplast and that AGPase was the major regulatory step in the pathway of starch synthesis (Preiss and Sivak, 1996). However, an increasing body of evidence shows, at least for barley, wheat, and maize, that an isoform of AGPase occurs in the cytosol, which accounts for most of the synthesis of ADPG and is less sensitive to regulation (Denyer et al, 1996b; Emes et al, 2003). Synteny among cereals could allow the assumption that, in rice also, ADPG is synthesized mainly in the cytosol and partly in the starch granule. The dual location of AGPase has at least two consequences: first, it raises interesting possibilities for the regulation of starch synthesis, and second, ADPG synthesized in the cytosol must be transported from the cytosol, across the amyloplast membrane into the starch granule, where it can be incorporated into starch.

Recent studies have identified a transporter for ADPG in the amyloplast envelope of wheat (Emes et al, 2003). The transporter catalyzes the exchange of ADPG with ADP, adenosine 5′-triphosphatase, and ATP, but it does not bind UDP glucose. Also, it is expressed in wheat endosperm from about 10 days after anthesis and thereafter is highly expressed during the rest of grain filling in wheat (Emes et al, 2003). Emes et al (2003) suggest that this protein could be the major route for the

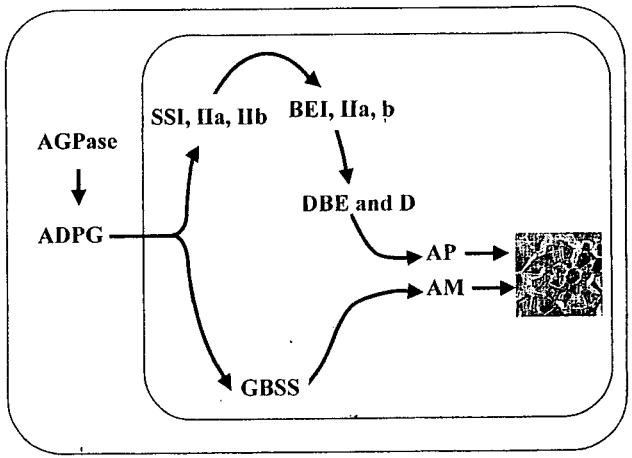

Fig. 4. Starch synthesis pathway. Adenosine 5′-diphosphatase (ADP) glucose pyrophosphorylase (AGPase) produces ADP-glucose (ADPG) in the cell but not inside the developing plastid. ADPG is transported into the plastid and then participates in the synthesis of amylose (AM) by granule-bound starch synthase (GBSS) or of amylopectin (AP) by the isoforms of starch synthase (SS) and branching enzyme (BE), and by the debranching enzyme (DBE) and the disproportionating enzyme (D).

CHAPTER 6

RICE PROTEINS

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Rice protein is an important source of nutrition and energy for 50% of the world's population, for whom rice has long been a staple diet. The protein content of rice, at approximately 7%, is relatively low compared with that of other cereal grains. However, because of the huge quantity of rice produced worldwide (approximately 400 million metric tons annually), the amount of rice protein potentially available is considerable. On the other hand, rice protein has a significant influence on the structural, functional, and nutritional properties of rice. It is a major factor in determining the texture (e.g., stickiness), pasting capacity, and sensory characteristics of rice. In recent years, rice protein has been recognized to be uniquely nutritious and hypoallergenic, which makes rice increasingly popular for use in foods all over the world.

Extensive research has been conducted on rice proteins because scientists recognize the importance of protein for the understanding and utilization of rice. However, only limited efforts have been made to keep up with and summarize the information on rice proteins in the literature. Earlier reviews on the subject include those of Houston (1972), Lasztity (1984), and Hamaker (1994). Of particular significance was the review by Juliano (1985) in the second edition of this book, a revision of his work (Juliano, 1972) in the first edition. A lot more research has been done since, particularly on the characterization of rice proteins, processing of rice protein products, and development of better-quality rice proteins. This chapter is an update of information in the literature on the chemistry and technology of rice proteins. It is an overview covering materials from sources old and new, but the emphasis is on studies reported since Juliano's review (Juliano, 1985).

PROTEIN DISTRIBUTION AND COMPOSITION

As in other cereal grains, the protein content of the embryo and aleurone layer in rice is as much as 20 percentage points higher than that of the endosperm. Nevertheless, the greatest part of the total proteins is located in the endosperm. Protein content usually is calculated from Kjeldahl nitrogen multiplied by the factor 5.95. This factor is based on the nitrogen content (16.8%) of the major rice protein, glutelin. Approximate protein contents of rough rice and its milling fractions are listed

TABLE 1
Proximate Protein Content of Rough Rice and Its Milling Fractions[a]

Rice Fraction	Crude Protein (g of N × 5.95)
Rough rice	5.6–7.7
Brown rice	7.1–8.3
Milled rice	6.3–7.1
Rice bran	11.3–14.9
Rice hull	2.0–2.8

[a] Source: Juliano (1993); reprinted with permission.

in Table 1. Values reported in the literature vary widely, depending on the variety, climate, and other agrotechnical conditions (Juliano, 1985). Invariably, milled rice contains lower quantities of protein than rough rice or brown rice because, during milling, a part of the protein-rich outer layers (aleurone cells) is removed. For the same reason, the protein content of the co-products of milling, such as the bran and the polish of the outer layers, normally is higher than that of the milled rice.

Protein Bodies

Up to 95% of the endosperm rice protein is in the form of discrete particles called protein bodies (PBs). They range in size from 1 to 4 μm, and mostly are concentrated in the peripheral-lateral and peripheral-dorsal cells (Fig. 1). There are at least two types of PBs in the rice endosperm, designated PB-I and PB-II. As observed under an electron microscope, PB-I displays a spherical shape and PB-II exhibits an irregular crystalline morphology (Bechel and Juliano, 1980; Tanaka et al, 1980; Ogawa et al, 1987, 1989). PB-I is highly enriched with prolamins and constitutes approximately 20% of milled rice protein. PB-II contains predominantly glutelins and constitutes 60–65% of milled rice protein. However, Barber et al (1998a), using polyclonal antibodies to purify protein fractions in immunocytochemical analyses, reported that the prolamins were not exclusively confined to PB-I or glutelins to PB-II. In studies on fecal particles of monogastric animals fed with rice proteins, the origin of the fecal protein particles remained unclear (Barber et al, 1998b). Intact PB-I and the fecal protein particle showed no significant difference in sizes, indicating that they might be the same protein particles. However, the residual particles contained immunocytochemically identified unique proteins that were also found in both PB-I and PB-II, raising questions about linking fecal protein particles exclusively with PB-I.

Protein Fractions

Osborne's classification of proteins based on solubility has been widely used in plant protein chemistry (Osborne, 1924). Essentially, proteins are separated into albumin (water-soluble), globulin (salt-soluble), prolamin (alcohol-soluble), and glutelin (alkaline-soluble). New approaches and techniques have shown that the distinction among the four Osborne fractions of plant proteins is not always straightforward and that the fractions are actually composed of mixtures of proteins (Juliano, 1985; Wilson, 1987). Nevertheless, the Osborne classification has provided pertinent solubility information and a solid basis for the studies of plant proteins. Typically, in traditional Osborne sequential extractions, ratios of albumin, globulin, prolamin, and glutelin have been reported to be in the range of 3.0–18.7:0–17:1.6–20.6:55.0–88.1 for brown rice and 0.9–9.9:1.4–19.9:0.4–10.3:61.8–91 for milled rice (Lasztity, 1984). These ratios and the range for each fraction vary widely, depending on the variety of rice and the extraction conditions. For instance, Huebner et al (1990) reported that, while the mean ratio of the salt-soluble (albumin plus globulin) prolamin to glutelin for 33 short, medium, long, and extra-long rice cultivars was 7:9:84, the salt-soluble fraction varied twofold (from 4 to 10%) and the prolamin content varied by more than threefold (from 5 to 18%). The ratios also may change depending on the maturity of the grain. Lower molecular weight subunits were found to have increased and higher molecular weight subunits decreased at 14, 30, and 15 days after flowering; the effect was particularly pronounced for the glutelin subunit, resulting in a significantly lower average for this protein fraction (Chrastil and Zarins, 1994).

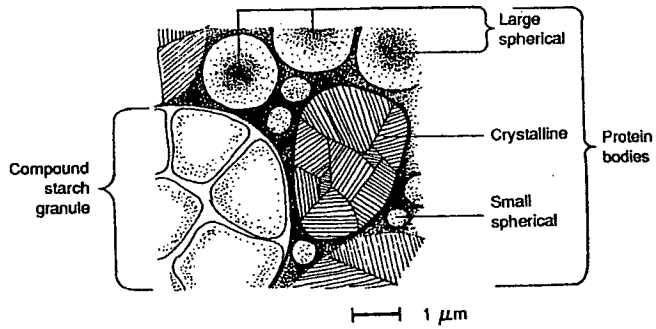

Fig. 1. Schematic diagram of various protein bodies and the compound starch granule in the endosperm subaleurone layer. (Reprinted, with permission, from Coffman and Juliano, 1987)

The approximate distribution of albumin in brown rice is bran, 31% (including germ, 13%); polish, 9%; and milled rice, 40% (Juliano, 1972). The corresponding distribution for globulin is bran, 40% (including germ, 10%); polish, 4%; and milled rice, 56%. The prolamin distribution is bran, 21% (including germ, 10%); polish, 4%; and milled rice, 75%. Approximately 5% of the glutelin is in the bran (principally in the germ), 2% in polish, and 93% in milled rice. The proportion of albumin in protein is highest in the outer layers of milled rice and decreases toward the center, whereas the proportion of glutelin has an inverse distribution (Houston et al, 1968).

A summary of the amino acid composition for milled rice protein fractions is shown in Table 2. Albumin has the highest lysine content, followed by glutelin, then globulin and prolamin. Globulin is richest in the sulfur amino acids cysteine and methionine; prolamin is the poorest. When brown rice protein content increases from planting or climatic conditions, it is due mainly to the increase in glutelin content (Cagampang et al, 1966; Souza et al, 1999). The slight increase in low-lysine prolamin may explain the decrease in the lysine content of protein as protein content increases.

(Chen and Chang, 1984; Shaw and Sheu, 1992). Shih and Daigle (2000) reported that treatment of this co-product with α-amylase and glucoamylase resulted in a product containing 85% protein. Follow-up treatment with a mixture of cellulase and xylanase raised the protein content to 92%. Inorganic impurities, particularly the metal manganese that was present at an unusually high level of 47 mg/kg in the starting rice flour, also were removed from the protein product. The removal of manganese is desirable because the consumption of high levels of manganese is known to cause liver problems and nerve system disorders in humans (Butterworth et al, 1995; Fell et al, 1996).

Functional Properties

Compared with other plant proteins, rice protein has relatively poor food-use functional properties (Barber and Barber, 1974). Rice proteins are extremely insoluble because of the intermolecular disulfide linkages and high molecular weights of the major protein glutelin (Cagampang et al, 1966; Sawai and Morita, 1968; Tecson et al, 1971). The solubility and, with it, other food-use functional properties are further reduced during processing, particularly by modifications such as heat treatment for processing or stabilization (Knorr, 1982; Knorr and Betschart, 1983; Prakash and Ramanatham, 1995a; Devi et al, 1997). Functional properties also can be influenced by the drying technique employed. Freeze-dried protein concentrates from both heat-stabilized and parboiled rice bran had low water-absorption capacity and high fat-absorption capacity. Cabinet-dried samples did not exhibit any foaming or emulsification capacity, indicating significant molecular denaturation (Prakash and Ramanatham, 1995a).

The protein in rice flour, though only about 7%, plays a significant role in determining the functional properties of the starch, which makes up approximately 80% of the rice kernel (Cheng, 1987; Marshall et al, 1990). The removal of protein by the treatment of buffer or protease (Fig. 3) tends to bring about a decrease in the gelatinization temperature of the flour (Marshall et al, 1990), indicating that rice protein has an inhibitory effect on the swelling of rice starch granules. Measuring the interaction of protein and starch in a model system, Chrastil (1990) found stickiness of whole cooked rice to be positively correlated with the binding ratios of the rice protein oryzenin to starch, amylose, and/or amylopectin. Cooked rice stickiness was reported to increase with the disruption of the disulfide linkages in the protein component (Hamaker and Griffin, 1990, 1993; Hamaker et al, 1991). Proteins from the outer layers of the rice kernel, tightly bound to the starch, were found to be responsible for reducing the pasting and crystallizing capacities of the starch (Yang and Chang, 1999). Protein content also has been reported to correlate negatively with the expansion ratio for gun-puffed milled rice or oil-puffed parboiled rice (Villareal and Juliano, 1987). It is negatively correlated with the peak viscosity and positively correlated with the pasting temperature of the isolated rice starch (Lim et al, 1999).

Utilization

In spite of their limited food-use functional properties, rice proteins have been successfully utilized in food. For years, with rice proteins as a key ingredient, rice flour or rice bran has been incorporated into foodstuffs such as bread, beverages,

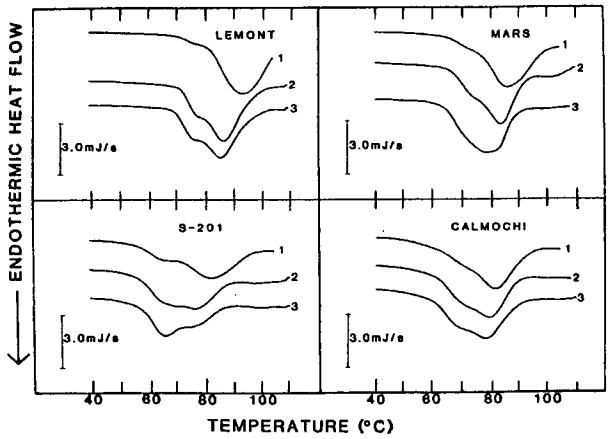

Fig. 3. Differential scanning calorimetry thermal curves of different whole grain milled rice varieties: untreated (curve 1), buffer-treated (curve 2), and Pronase-treated (curve 3). The water content was 70% (w/w), and the heating rate of the calorimeter was 1.0°C/min. (Reprinted, with permission, from Marshall et al, 1990)

pasta, and confections (Lynn, 1969; Carroll, 1990; Saunders, 1990). Recently, as high-protein rice products have become more available, the use of rice proteins in food has surged. They have been used in infant foods (Hanson et al, 1981), breakfast cereal (Bakar and Hin, 1985), snack foods (Capansana et al, 1984), and edible films (Shih, 1996). To meet the needs of hypoallergenic protein products, a rice product developed by enzymatically removing the allergic factor in the protein component has been produced and made commercially available in Japan (Watanabe, 1993).

NUTRITIONAL PROPERTIES

Percent protein in rice generally is calculated based on the Kjeldahl conversion factor of 5.95. However, in nutritional studies, the factor 6.25 is used to maintain a common nitrogen-based calculation for all proteins. Rice protein is nutritionally important to many people. In tropical Asia, in particular, rice is the principal source of the dietary protein (35–40%) and energy (50%) of the population (Juliano, 1993). Protein-intake studies showed that intakes of 0.94–1.23 g/kg per day, at energy levels of 37–63 kcal/kg per day, were adequate for Chilean, Chinese, Filipino, Korean, and Thai subjects (Intengan et al, 1984; Rand et al, 1984). Based on the safe level of protein requirement for milk of 0.89 g/kg of body weight (Huang et al, 1980), the high-protein rice (IR58) had 62% of the protein quality of milk. In general, rice protein, like most cereal proteins, is deficient in the essential amino acid lysine, but it has an excess of the essential sulfur-containing amino acids cysteine and methionine. By contrast, legume proteins, such as soy protein, are deficient in sulfur

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CHAPTER 7

RICE LIPIDS

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Lipids in rice, although not as abundant as the carbohydrate and protein components, are important because they contribute to nutritional, sensory, and functional qualities. Lipid rancidity in brown rice is well known as a major deterrent to more widespread food usage of this form of rice. In addition, lipids associated with the starch component have been found to affect the pasting and other functional characteristics of starch. Recent evidence for the contribution of minor lipid components of rice to protection against chronic diseases such as heart disease and cancer have heightened interest in rice lipids from a nutritional standpoint. This chapter characterizes the lipids in rice, with a focus on specific categories and classes of lipids and emphasis on their functionality.

Several reviews have been published on the chemistry of rice lipids (Juliano, 1977, 1983; Fujino, 1978; Morrison, 1978), although little additional information has been published on the gross composition of rice lipids since the publication of the previous edition of this text. This edition includes recent developments in rice lipid composition, with emphasis on factors that affect that composition. It also includes recent information on the nutritionally important minor constituents.

Lipids are present in the form of spherosomes, or lipid droplets, with diameters of <1.5 μm in the aleurone layer, <1 μm in the subaleurone layer, and <0.7 μm in the embryo of rice grain (Juliano, 1983). In addition, endosperm cell walls stain for lipid dyes such as Sudan IV (Yoshizawa et al, 1980). Most of the lipids in the endosperm are associated with protein bodies, but it is believed that starch granules also have bound lipids.

Lipids are generally classified into nonstarch lipids, principally those in the spherosomes of the aleurone layer and embryo, and starch lipids (Morrison, 1978). The former represent the majority of the lipids present in rice, but the latter have considerable importance relative to starch functionality (Morrison, 1995). Morrison (1981) proposed the term *starch surface lipids* for nonstarch lipids that contaminate internal starch lipids (i.e., lipids inside the granule), but Gailliard and Bowler (1987)

contended that lipids found on the surface of the granule should be considered starch lipids. With current interest in the functionality of starch and the potential role of lipids in functionality, additional efforts have been made to further clarify the origin and roles of lipids associated with starch, as discussed later.

EXTRACTION OF LIPIDS

Nonpolar solvents such as diethyl ether, petroleum ether, and chloroform/methanol (2:1, v/v) readily extract nonstarch lipids from dry grain (Morrison, 1978; Choudhury and Juliano, 1980a). Residual nonstarch lipids may be further extracted with water-saturated butanol (WSB) for 30 min to 1 hr at 20–30°C. Starch granules damaged as a result of milling steps, unlike undamaged granules, leach out starch lipids into WSB at ambient temperature and contaminate the nonstarch lipids.

Lipids of protein bodies of raw milled rice were 84% extracted by 8-hr contact with chloroform/methanol (2:1, v/v) at 25°C; an 11% additional extraction by 5-min contact with cold WSB produced a total of 95% extraction (Choudhury and Juliano, 1980a). Thus, protein body lipids would mostly be in the nonstarch lipid fraction of brown or milled rice.

Starch lipids may be extracted with WSB after extraction of nonstarch lipids. Although WSB gave an appreciable yield of internal wheat starch lipids from five overnight extractions at 2–4°C, an estimated 2.5×10^5 hr would be needed to extract all internal rice starch lipids under these conditions (Morrison, 1981). Extraction of pronase-digested rice flour with WSB twice for 12 hr each at ambient temperature removed 83–85% of the lipids from waxy rices and 50–86% of the lipids from nonwaxy rices. For a sample of IR42 brown rice from which the nonstarch lipids had been extracted, further extraction by cold WSB reduced fat-by-hydrolysis from 0.41 to 0.09%, indicating a 78% extraction of starch lipids by cold WSB (Choudhury and Juliano, 1980a). Refluxing WSB (67% butanol and 33% water at 92°C), which gelatinizes the starch granules, is required to completely extract starch lipids. Refluxing 95% ethanol was only as effective as cold WSB in extracting lipids from rice starch (IRRI, 1983a). Azudin and Morrison (1986) contended that the most appropriate method to obtain starch lipid quantification would be to extract all lipids from finely milled rice with hot propanol-water and extract total lipids from purified starch in the same manner, with the difference being nonstarch lipid.

MAJOR LIPID CATEGORIES

Starch Lipids

Starch lipids represent a relatively small proportion of the total lipid composition of rice but may play a role in starch synthesis and appear to make a major contribution to starch functionality (Morrison, 1995). As mentioned previously, recognition of the difficulties in separating lipid associated with the starch in the granule from lipid that occurs incidentally on the surface of the starch granule has been the subject of considerable debate and study. Lipids can be associated with starch in several different ways (Morrison, 1988). Morrison (1988) suggested that the classification system used for wheat starch may be applicable for all starches. In this system, lipids inside the starch granule would be considered true starch lipids. Lipids from the surrounding proteinaceous matrix of the endosperm would be classified as starch surface lipids. Both of these categories are composed primarily of monoacyl lipids. All other lipids associated with starch would be called nonstarch lipids (Morrison, 1988). Vasanthan and Hoover (1992) reported levels of lipid in what they termed "highly purified starch granules," in which the degree of starch damage was also assessed. They classified starch lipids as total lipid by acid hydrolysis, unbound lipid by chloroform/methanol (2:1, v/v), and bound lipid by hot *n*-propanol/water (3:1, v/v), with the unbound lipid being considered primarily surface lipid. Kitahara et al (1997) employed glucoamylase as a means to more appropriately characterize lipids associated with starch, and recent use of more sophisticated spectroscopic techniques has further clarified starch-lipid interactions (Morrison et al, 1993). However, the exact nature of starch-associated lipid has not been entirely delineated.

Starch lipids extracted with cold WSB from defatted brown rice amounted to 0.6–0.7% for nonwaxy rice and 0.2% for waxy rice (Choudhury and Juliano, 1980b). Corresponding values for milled rice were 0.5% for nonwaxy and 0.1% for waxy rice. Since cold WSB extracted only 78% of brown-rice starch lipids relative to hot WSB, the true starch lipid level would be 0.7–0.8% for nonwaxy rice. Thus, nonwaxy rice has more starch lipid and less nonstarch lipid in brown and milled rice than does waxy rice. However, the total content of grain lipids seems to be similar. IR42 brown rice (29% amylose) had 2.5% nonstarch lipids and 0.7% starch lipids; IR480-5-9 brown rice (24% amylose) had 2.7% nonstarch lipids and 0.8% starch lipids; and IR4445-63-1 (waxy) had 2.9% nonstarch lipids and 0.2% starch lipids, for a total lipid content of 3.1–3.5% at 14% moisture. Corresponding values for over-milled rice were 0.4% nonstarch plus 0.6% starch lipids for IR24 and IR480-5-9, and 0.7% plus 0.1% for waxy rice, for a total of 0.8–1.0% total lipids. Kawashima and Kiribuchi (1980) reported 0.6% nonstarch and 0.6% starch lipids in a nonwaxy milled rice and 0.8% nonstarch and 0.4% starch lipids in a waxy milled rice (a total of 1.2%).

Morrison and Azudin (1987) reported a wide range of starch lipids, from less than 0.1% in waxy varieties to as high as 1.3% in nonwaxy varieties. These researchers evaluated the influence of genetics and environmental conditions on starch lipids and looked for a potential correlation between starch lipids and amylose content. For some varieties, a clear relationship was seen between amylose and starch lipid but not in all cases.

Starch lipids consist primarily of lysophospholipids and free fatty acids (Morrison, 1988). Neutral lipids and glycolipids have been reported (Choudhury and Juliano, 1980b), but these may be contaminants from nonstarch lipid (Morrison, 1988). Major phospholipid species were lysophosphatidylethanolamine and lysophosphatidylcholine. Starch lipids of waxy milled rice had more free fatty acids and fewer lysophospholipids than starch lipids of nonwaxy milled rice (Choudhury and Juliano, 1980b). Hirayama and Matsuda (1973) reported that lysophosphatidylcholine was the major phospholipid of starch lipids, followed by lysophosphatidylethanolamine, and that sterol glycosides were the major glycolipids.

Major fatty acids of nonwaxy milled-rice starch lipids were palmitic and linoleic, together with a lesser amount of oleic acid (Choudhury and Juliano, 1980a,b). Starch lipids had less oleic acid and more palmitic acid than nonstarch lipids. Starch lipids from waxy milled rice had 47% palmitic, 18% oleic, and 30% linoleic acid (Choudhury and Juliano, 1980b). Kawashima and Kiribuchi (1980) also reported starch lipids of milled rice to be 35% palmitic, 14% oleic, and 46% linoleic in non-

waxy rice and 38% palmitic, 15% oleic, and 36% linoleic in waxy rice. The neutral fraction of starch lipids from nonwaxy milled rice had 21–25% palmitic, 9–22% oleic, and 48–68% linoleic acid; glycolipids had 45–56% palmitic, 5–9% oleic, and 26–45% linoleic acid; and phospholipids had 46–50% palmitic, 5–16% oleic, and 30–42% linoleic acid (Choudhury and Juliano, 1980b; Maniñgat and Juliano, 1980). None of the three fractions had a fatty-acid composition similar to that of the total starch lipids.

The quantification of fat-by-hydrolysis probably corresponds to the fatty-acid fraction of starch lipids (Choudhury and Juliano, 1980a). Both had identical fatty-acid compositions. Cold-WSB extraction of 0.57% starch lipids from IR42 defatted brown rice reduced fat-by-hydrolysis from 0.35 to 0.08%, and the fat-by-hydrolysis corresponded to 48% by weight of starch lipids.

Influence of Starch Purification

The level and composition of the lipids of purified starch granules depends to a large measure on the method of preparation. Dodecyl benzene sulfonate (DoBS) extraction of protein during starch preparation of IR480-5-9 milled rice reduced cold-WSB lipids of starch from 0.69 to 0.38% and changed the neutral lipid-glycolipid-phospholipid ratio from 26:16:58 (Choudhury and Juliano, 1980b) to 30:36:34 (Maniñgat and Juliano, 1980). For eight milled rices, hot-WSB lipids ranged from 0.09 to 0.79% for DoBS-prepared starch, as compared to 0.19–1.42% for starch prepared by alkali protease (Maniñgat and Juliano, 1980; IRRI, 1983b). The corresponding cold-WSB lipids were 0.03–0.38% and 0.17–0.81% at 14% moisture. The major neutral lipid species of DoBS-prepared starch was free fatty acid (88–97%), with minor amounts of monoglycerides, diglycerides, and sterols. Major glycolipids were diglycosyl monoglycerides and monoglycosyl monoglycerides, together with smaller amounts of sterol glycosides and contaminant DoBS. The major phospholipid was lysophosphatidylcholine (61–91%), followed by lysophosphatidylethanolamine, with smaller amounts of their parent phospholipids. Ito et al (1979) reported 0.59% starch lipids for a DoBS-prepared starch consisting of 32% free fatty acids, 30% lysophosphatidylcholine, 11% monoglycosyl monoglycerides, 8% diglycosyl monoglycerides, 7% lysophosphatidylethanolamine, and 3% monoglycerides, equivalent to a fraction ratio of 37:20:43. Component sugars of glycolipids were predominantly galactose, plus glucose. DoBS-prepared starch had no nonstarch lipids extractable with petroleum ether (Maniñgat and Juliano, 1980).

A DoBS-prepared rice starch contained 47% palmitic, 9% oleic, and 40% linoleic acid in its lipids (0.59% at 14% moisture) (Ito et al, 1979). Free fatty acids were 30% palmitic, 10% oleic, and 54% linoleic acid; lysophosphatidylcholine had 79% palmitic, 7% oleic, and 9% linoleic acid; and lysophosphatidylethanolamine had 51% palmitic, 4% oleic, and 42% linoleic acid. Starch lipids from waxy rice prepared by the DoBS method had 58% palmitic, 19% oleic, and 14% linoleic acid (Maniñgat and Juliano, 1980).

The lipid fractions of two DoBS-prepared starches also showed differences in fatty-acid composition but were not identical to the corresponding fractions of starch lipids from milled rice (Maniñgat and Juliano, 1980). Neutral lipids had 24 or 34% palmitic, 11 or 12% oleic, and 62 or 50% linoleic acid; glycolipids had 37 or 41% palmitic, 3% oleic, and 58 or 52% linoleic acid; and phospholipids contained 57 or 63% palmitic, 4% oleic, and 35 or 26% linoleic acid.

In contrast to the neutral lipid-glycolipid-phospholipid ratio for starch lipids extracted with cold WSB from IR480-5-9 brown rice (28:20:52) and from milled rice (26:15:59) (Choudhury and Juliano, 1980b), starch prepared by alkaline protease treatment had 0.7% cold-WSB lipids with a ratio of 44:7:49 uncorrected for contaminant nonstarch lipids (Maniñgat and Juliano, 1980). Sawada and Mano (1980) reported ratios of 51:15:34 and 33:9:58 for two protease-prepared tropical rice starches. Mano (1982) reported a ratio of 37:20:43 for DoBS-prepared starch of a Japanese variety. Azudin and Morrison (1982, *unpublished data*) reported 0.02% free fatty acids and no lysophospholipids as true starch lipids in two waxy rice starches and 0.14–0.30% free fatty acids and 0.45–0.64% lysophospholipids as true lipids for six nonwaxy rice starches, equivalent to a ratio of 27–40:0:60–73 (mean ratio, 33:0:67). Free fatty acids, lysophosphatidylcholine, and lysophosphatidylethanolamine were the major hot-WSB starch lipids of two nonwaxy starches; the amounts were 34, 23, and 10% and 36, 18, and 18%, respectively (Sawada and Mano, 1980).

Contaminant nonstarch lipids in protease-prepared rice starches were 0.75 and 0.98% in two waxy rices at 14% moisture (0.02% starch lipids) and 0.45–1.08% in six nonwaxy starches with 0.65–0.93% starch lipids (Azudin and Morrison, 1982, *unpublished data*). The mean content was 0.68% nonstarch lipids and 0.77% starch lipids. In waxy rice starch, major nonstarch lipids were triglycerides, diglycerides plus free fatty acids, lysophospholipids, sterol esters, monoglycerides plus acyl sterol glycosides, and glycosyl glycerides. Among the six nonwaxy starches, the major type of nonstarch lipid was triglycerides, followed by lysophospholipids, diglycerides plus free fatty acids, and variable amounts of monoglycerides plus acyl sterol glycosides, sterol esters, and glycosyl glycerides.

Alkali treatments to prepare starch probably saponify starch lipids and render them extractable as soap (sodium salt of fatty acids). Thus, Schoch (1942) extracted soap from commercial rice starch with 0.59% lipids, and the residual lipid content after seven 1-hr extractions with three parts of 85% methanol under reflux was 0.03% (95% extraction); it was 0.07% (88% extraction) after 48 hr of Soxhlet extraction with 80% dioxane in water. Ohashi et al (1980) removed >60% of starch lipids by 3-hr reflux with five parts of 99% methanol; these lipids consisted of 44 mol% linoleic, 37 mol% palmitic, and 13 mol% oleic acid. A starch prepared with 0.1% NaOH had 0.71% fat-by-hydrolysis at 14% moisture and 0.71% hot-WSB starch lipids (Ohashi et al, 1980).

The 0.1% NaOH-prepared rice starch had starch lipids consisting of 70% free fatty acids plus diglycerides, 22% lysophospholipids (mainly lysophosphatidylcholine), 4% diglycosyl glycerides, 3% monoglycerides plus acyl sterol glycosides, 2% each of monoglycosyl glycerides and sterol esters, and <1% triglycerides (Azudin and Morrison, 1982, *unpublished data*). The neutral lipid-glycolipid-phospholipid ratio was approximately 72:6:22. Alkali treatment to remove protein reduced cold-WSB lipids of IR480-5-9 rice from 0.69 to 0.24% for 0.1% NaOH and to 0.08% for 0.2% NaOH, with corresponding lipid fraction ratios of 80:9:11 and 79:9:12, respectively (Maniñgat and Juliano, 1980), using a milled rice with a starch lipid ratio of 26:16:58 (Choudhury and Juliano, 1980a). Rice starch prepared with 0.1% NaOH had 0.13% nonstarch lipids in addition to 0.47% starch lipids (N. S. Azudin and W. R. Morrison, 1982, *unpublished data*). Major nonstarch lipids were 91% free fatty acids plus diglycerides and 6% triglycerides.

Vasanthan and Hoover (1992) evaluated the lipid composition of a commercially prepared rice starch (Sigma Chemical Co., St. Louis, MO) using their system of

classifying lipids as total, bound and unbound. They found total lipid, by acid hydrolysis, to be 0.76%, of which 0.05% was unbound and 0.71% was bound. Neutral lipid was the predominate class of lipid in the unbound, or surface lipids, whereas phospholipids were the predominate class in the bound lipids. Hoover et al (1996) found 0.68% total lipid, with 0.17% unbound and 0.50% bound in long-grain brown rice (IR64).

Nonstarch Lipids

Nonstarch lipids are found in the aleurone, subaleurone, and germ of brown rice, and a small amount is in the hull of rough rice. Obviously, the type and degree of milling influences the content of nonstarch lipid in rice products. In fact, it has been suggested that degree of milling could be determined based on grain-surface lipid quantification (Bennett et al, 1993; Chen et al, 1998).

The hull of IR42 rice had about 0.4% lipid (nonstarch) at 14% moisture and contributed little to total rough rice lipids (Choudhury and Juliano, 1980a). It contributed 0.4% of total nonstarch lipids (0.3% of total neutral lipids, 17% of total glycolipids, and 7% of total phospholipids) of rough rice and consisted mainly of neutral lipids (Table 1). Hexane-extracted hull lipids had similar fatty acids as brown-rice nonstarch lipids (Hartman and Lago, 1976) (Table 1), but it contained <1% arachidic ($C_{20:0}$), <2% behenic ($C_{22:0}$), and <4% lignoceric ($C_{24:0}$) acids (Morrison, 1978).

Using ^{13}C nuclear magnetic resonance spectroscopy, Bradbury and Collins (1982) confirmed a lipid content of 3.1% for IR480-5-9 brown rice, of which 0.75% was for endosperm, 27.3% for germ, and 26.7% for aleurone cells plus grain coat at 14% moisture, which is equivalent to 22% of brown-rice nonstarch lipids each in endosperm and germ, and 56% in aleurone cells plus grain coat.

In three brown-rice samples, nonstarch lipids at 14% moisture were 2.5% for 29%-amylose rice, 2.7% for 24%-amylose rice, and 2.9% for waxy (1.7%-amylose) rice (Choudhury and Juliano, 1980b). The difference in fat content may be due in part to the higher nonstarch lipid content of the inner endosperm, which makes up 85% of brown rice. The nonstarch lipid content was 0.7% of the inner endosperm of waxy rice, as compared with 0.4% for the two nonwaxy rices. Reported nonstarch lipids for Japanese brown rice are 2.4% (Hirayama and Matsuda, 1973) and 2.6% (Fujino and Mano, 1972). The mean lipid content of brown rice from cultivars grown in the United States was 2.87% (n = 14) for long-grain type, 2.76% (n = 10) for medium-grain type, 2.49% (n = 2) for short-grain type, and 3.11% (n = 2) for short-grain (waxy) type (Taira and Itani, 1988).

Nonstarch lipids of brown rice had saponification number, iodine number, and unsaponifiable matter content mostly similar to those of its fractions (Table 1). Rice hull lipids differ from brown rice lipids in saponification number and iodine number, probably because of their higher level of unsaponifiable matter (Hartman and Lago, 1976) and the lower linoleic acid content (Table 1). Major fatty acids are 36–38% linoleic ($C_{18:2}$), 35–37% oleic ($C_{18:1}$) and 23–24% palmitic ($C_{16:0}$) acids (Choudhury and Juliano, 1980a,b). The higher level of palmitic acid and lower level of oleic acid in milled-rice nonstarch lipids may be due in part to contaminant starch lipids. Minor fatty acids are myristic ($C_{14:0}$), stearic ($C_{18:0}$), and linolenic ($C_{18:3}$), plus a trace of lauric ($C_{12:0}$), 0.1–0.5% palmitoleic ($C_{16:1}$), and 0.3–0.7% arachidic acids (Lugay and Juliano, 1964). Tanaka et al (1982) found 0.4–0.5% gadoleic ($C_{20:1}$),

TABLE 1
Mean Composition of Lipids of Hull, Brown Rice, and Its Fraction[a,b]

	Nonstarch Lipids in Rice Fractions						Nonwaxy Starch Lipids	
Property	Hull	Brown	Milled	Bran	Germ	Polish	In Brown Rice	In Milled Rice
Lipid content, wt %	0.4	2.7	0.8	18.3	30.2	10.8	0.6	0.5
Saponification number	145	181	190	184	189	...	...	...
Iodine number	69	94	100	99	101	...	...	...
Unsaponifiable matter	26	6	6	6	34	...	...	...
Fatty acid composition,[c] wt % of total								
Palmitic	18	23	33	23	24	23	46	45
Oleic	42	35	21	37	36	35	12	11
Linoleic	28	38	40	36	37	38	38	40
Others[d]	12	4	6	4	3	4	4	4
Neutral lipids,[c] % of total lipids	64	86	82	89	91	87	28	26
Triglycerides	...	71	58	76	79	72	4	2
Free fatty acids	...	7	15	4	4	5	20	21
Glycolipids, % of total lipids	25	5	8	4	2	5	19	16
Phospholipids, % of total lipids	11	9	10	7	7	8	53	58
Phosphatidylcholine	...	4	4	3	3	3	4	4
Phosphatidylethanolamine	...	4	4	3	3	3	5	5
Lysophosphatidylcholine	...	<1	2	<1	<1	<1	21	23
Lysophosphatidylethanolamine	...	...	1	...	...	...	22	25

[a] Based on 6% bran-germ, 4% polish, and 90% milled rice from brown rice.
[b] Data from Juliano (1977), Choudhury and Juliano (1980a,b), Hartman and Lago (1976), and Yusta and Santos (1953).
[c] Composition of mean of two nonwaxy rices and one waxy rice for nonstarch lipids, of the two nonwaxy rices only for nonwaxy starch lipids (Choudhury and Juliano, 1980b), and of IR42 only for hull (Choudhury and Juliano, 1980a).
[d] Trace to 3% myristic, 2–4% stearic, and 1–2% linolenic.

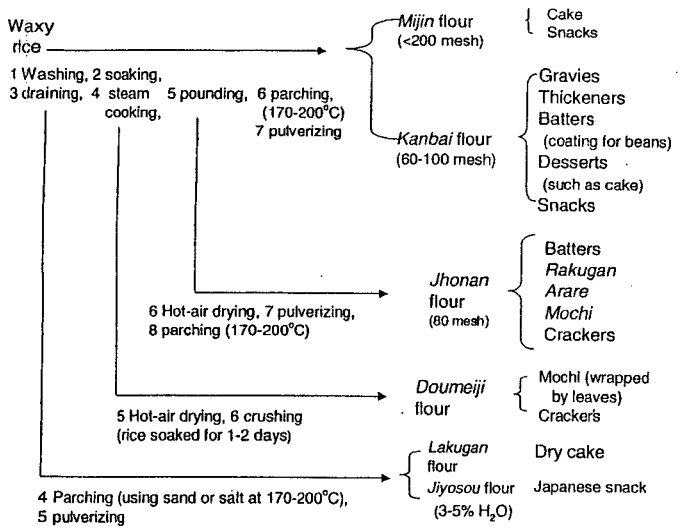

Fig. 7. Manufacture and applications of precooked waxy specialty rice flours.

polymerization (Doublier et al, 1986). Delrue and Chamberlin (2000) used a continuous process to prepare partially gelatinized rice flour with low moisture content (less than 30%) at low temperature (about 74°C) using steam.

FUNCTIONAL PROPERTIES

Effect of Inherent Properties

Because rice flours are made from polished rice or brokens, their chemical composition should be the same or similar to that of whole rice (*Oryza sativa* L.). The differences among rice flours are governed by inherent cultivar variations, methods of milling or grinding, and the pretreatments of rice or flour. Rice flour is identified by the variety or the amylose content, which indicates its inherent properties. As discussed in previous chapters, rice can be classified traditionally based upon the original planting area. Indica rice has been grown in India, Sri Lanka, Pakistan, Bangladesh, Taiwan, southern China, Thailand, Vietnam, Burma, Laos, Cambodia, etc. Japonica rice is planted in the northern and central regions of China, Korea, and Japan. Javanica rice is bred in Indonesia. The grain length is a common standard with which to identify rice type in the United States. Three common types are long (longer than 6.6 mm), medium (between 5.5 and 6.6 mm), and short (shorter than 5.5 mm). Sometimes the grain longer than 7.5 mm is called extra long. Endosperm characteristics are also used for classification. Waxy (or glutinous or sweet) rice consists of less than 1% amylose, and nonwaxy rice generally has an

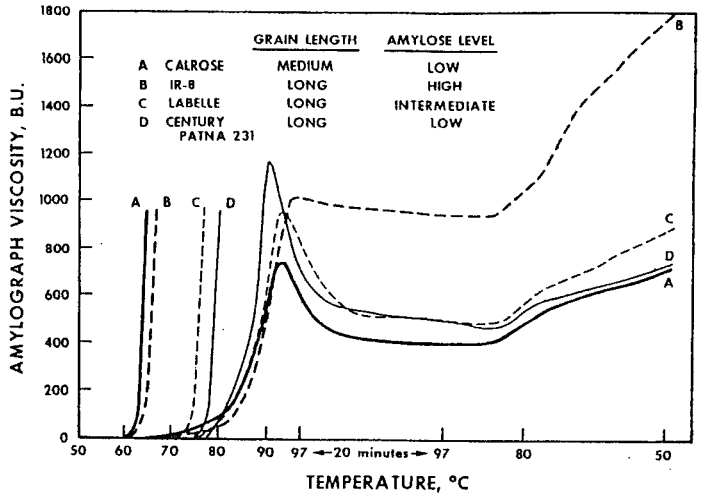

Fig. 8. Amylograph curves for four rice varieties. Short curves, 20% slurries; full curves, 10% slurries. (Reprinted, with permission, from Nishita and Bean, 1979)

amylose content higher than 10%. In general, indica rice or long-grain rice has a high amylose content and gelatinization temperature. Japonica rice or short-grain rice contains an intermediate level of amylose. It appears that the amylose content plays a key role in the inherent properties of rice flour. Various methods are used to determine the amylose content in starch. The iodine titration method developed by Juliano et al (1981) is generally used for rice.

Rice starch granules are the smallest (3–10 μm) among cereal starches. Amylose content or amylose-amylopectin ratio, gelatinization, and pasting behavior are the important properties for describing rice starch. Amylography is usually used to analyze the pasting behavior of rice flour or starch. Figure 8 shows two sets of pasting curves for four cultivars having contrasting properties (Nishita and Bean, 1979). The full curves, which include heating, holding, and cooling modes, use 10% rice flour slurries. The short curves on the left are obtained with 20% slurries. At this high concentration, a rapid increase in measurable viscosity occurs immediately upon the onset of granule swelling. The temperature at which a short curve leaves the baseline gives a fairly reliable estimate of gelatinization temperature. Juliano et al (1985) estimated gelatinization temperatures from the intercept of short curves with the 20-BU line.

In Figure 8, sample A is Calrose, a California medium-grain with low amylose content and low gelatinization temperature. Sample C is Labelle, a typical long-grain rice with both intermediate amylose content and gelatinization. Sample D, Century Patna, is one of the few atypical U.S. rices. It has long-grain length but low amylose content and high gelatinization temperature, both characteristics outside those for a standard U.S. long-grain rice. Sample B, IR-8, is a long-grain rice with high amylose

content but low gelatinization temperature. It is also atypical by U.S. standards. The pasting curve for IR-8 shows negligible viscosity breakdown during a high-temperature holding cycle and a marked viscosity increase during the cooling cycle, indicating that retrogradation is rapid (Bean, 1986). It appears that the grain length alone is not adequate to represent the properties of rice.

Researchers and industrial users of rice flour have adapted other amylograph methods. Variations include changes in slurry concentration, starting temperature, heating rate, and endpoint temperature. They are usually designed to reflect processing conditions for individual products, and hence they predict functional performance of the rice flour for a specific application. Sharp (1986) developed a rapid procedure for determining amylograph viscosity of rice flour. The results have a linear relationship with the standard method. A Rapid ViscoAnalyser (RVA) has been used to determine the viscosity of rice flour (Blakeney et al, 1991) using a 3-g sample instead of the 27- to 28-g sample used in the standard amylograph method. The test can be completed in 15 min instead of the more than 20 min required for the standard amylograph procedure. Due to the convenience and small quantity of sample, the RVA has been widely adopted.

Differential scanning calorimetry (DSC) has been widely used to determine the temperature and heat of gelatinization. The water content affects the thermal behavior of rice starch. Biliaderis et al (1986) demonstrated that a single symmetrical endotherm accounts for the phase transition of starch isolated from rice, IR-8, when water content is higher than 60% (Fig. 9). As the water content decreases, the size of the transition endotherm is progressively reduced, with the concomitant development of a second transition at a higher temperature. The authors also pointed out that the correlation between DSC data and birefringence endpoint temperature (BETP) was significant ($r = 0.84$, $P < 0.01$). By combining light microscopy with DSC, Yeh and Li (1996) confirmed that two stages of swelling and disruption/dissolution of starch granules are involved during gelatinization. The peak temperature (T_p) of the DSC thermogram is the transition point for the two stages. The rate of granule rupture reaches maximum at T_p (Fig. 10). When DSC is used to determine the thermal behavior of starches, the energy transferred from the surroundings to the system, measured by DSC, can be expressed as a function of enthalpy and pressure as follows:

$$\Delta Q = \Delta H - V\Delta P$$

where ΔQ is the energy transferred from the surroundings to the system, ΔH is the change in enthalpy, V is the volume, and ΔP is the change in pressure. The change in energy determined by DSC is ΔQ. When the system is at constant pressure (i.e., $\Delta P = 0$), then $\Delta Q = \Delta H$. However, it is very difficult to ensure that the pressure in the crucible or sample pan is constant during DSC measurement, particularly when the water vapor pressure exceeds 1 atm at temperatures higher than 100°C. Therefore, it is more accurate to describe the endothermic energy of a starch solution measured by DSC as the "heat" instead of "enthalpy" of gelatinization.

Rice flours and their isolated starch counterparts have been found to exhibit similar gelatinization characteristics (Chungcharoen and Lund, 1987) in the DSC thermogram. The presence of sucrose or sodium chloride results in a shift of gelatinization to higher temperature (Table 1), and heat associated with the endothermic process decreases. The effect of solutes on gelatinization is more remarkable at low water content. Among the tested solutes, the surfactant con-

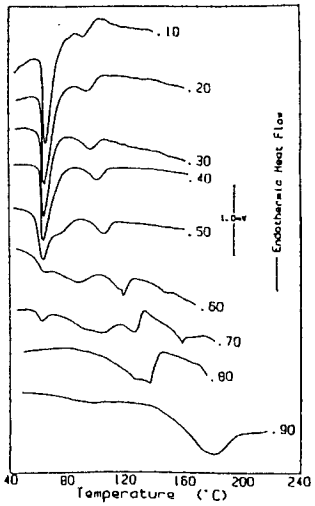

Fig. 9. Differential scanning colorimetry thermal curves of IR-8 rice at various water contents. Numbers designate the weight fraction of starch. Starch weight from top to bottom (mg): 1.08, 2.03, 3.22, 4.03, 5.09, 5.41, 4.53, 5.61, and 3.96. Heating rate 10°C/min. All data files were normalized to a constant sample weight of 5 mg. (Reprinted, with permission, from Biliaderis et al, 1986; ©American Chemical Society)

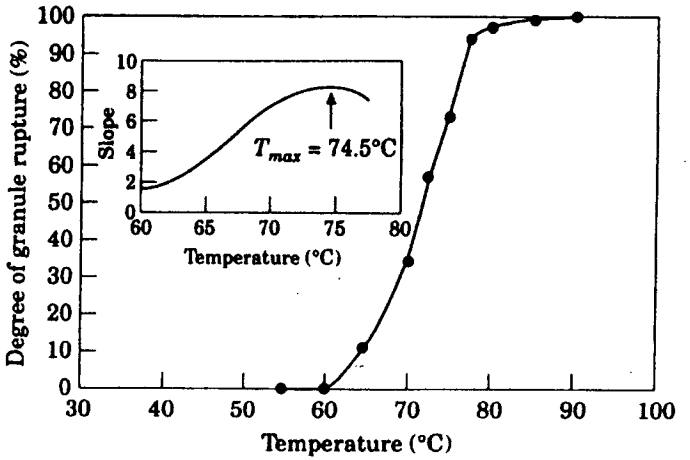

Fig. 10. Degree of granule rupture of rice starch at different temperatures as observed microscopically. (Reprinted, with permission, from Yeh and Li, 1996)